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(57) Abstract

Photoremovable protecting groups for the creation of large scale chemical diversity are disclosed. Orthonitrobenzyl groups containing various phenyl-ring substituents, benzylic carbon substituents, and benzylic carbon extensions protect nucleoside and amino acid functional groups, including hydroxyl, amino, and carboxyl groups. Phenyl-ring substituents include cyclic orthoesters, acetals, ketals, substituted amines, and cyclic ethers.

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NOVEL PHOTOREACTIVE PROTECTING GROUPS

FIELD OF THE INVENTION

The present invention relates to the field of photosensitive protecting groups. More specifically, the invention provides ortho-nitrobenzylic groups for protecting carboxylic, amino, thiol, hydroxyl, and other moieties.

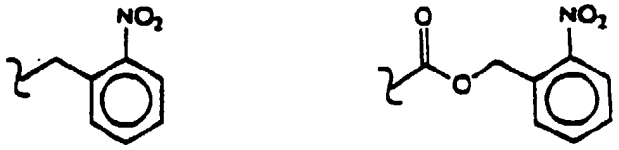
BACKGROUND OF THE INVENTION

Chemical protecting groups are used during synthesis reactions to temporarily protect certain functional groups on a compound against undesired reactions. When a reaction sequence is complete, and protection is no longer necessary, the protective group is removed to restore the protected functional group to its natural activity. Protective groups are removed by various procedures such as exposure to acidic or basic conditions. One class of protective groups, the photolabile or photosensitive protecting groups, are removed by exposure to electromagnetic radiation of a prescribed wavelength.

The properties and uses of some photolabile protecting compounds have been reviewed. See, McCray et al., Ann. Rev. of Biophys. and Biophys. Chem. (1989) 18:239-270, which is incorporated herein by reference. A particularly useful class of photoremovable protecting groups is the ortho-

nitrobenzyl derivatives. When exposed to radiation (typically near ultra-violet), these compounds undergo an internal rearrangement that results in deprotection of the protected compound. In the process, the protected compound splits off an ortho-nitroso aldehyde or ketone, leaving a free hydroxyl, amino, or other previously protected group. See Cameron and Frechet, J. Am. Chem. Soc. (1991) 113:4303-4313, incorporated herein by reference for all purposes.

Examples of known ortho-nitrobenzyl photoremovable protecting groups are described in, for example, Patchornik, J. Amer. Chem. Soc. (1970) 92:6333, Amit et al., J. Org. Chem. (1974) 39:192, Pillai, Synthesis (Jan. 1980) 1-26, and E.P. Application 046 083, all of which are incorporated herein by reference. Known members of this class include ortho-nitrobenzyl and ortho-nitrobenzyloxycarbonyl protected compounds.

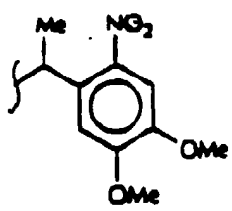


Patchornik and others have reported some phenyl-substituted ortho-nitrobenzyl groups for protecting amino acid functional groups. For example, the phenyl ring has been substituted methoxy groups at the 3 and 4 positions (nitroveratryl). In other known compounds, one of the benzylic hydrogens has been replaced with a methyl or phenyl (substituted and unsubstituted) group.

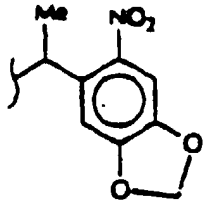
Ortho-nitrobenzyl protected compounds have now found use in a variety of fields. For example, they have proven useful in Very Large Scale Immobilized Polymer Synthesis (VLSIPS™) which provides techniques of forming vast arrays of peptides and other polymer sequences using, for example, light-directed synthesis techniques. The details of VLSIPS™ are disclosed in Pirrung et al., U.S. Patent No. 5,143,854 (see also PCT Application No. WO90/15070), Fodor et al., PCT Publication No. WO92/10092, and Fodor et al., Science (1991)

251:767-777, all incorporated herein by reference for all purposes.

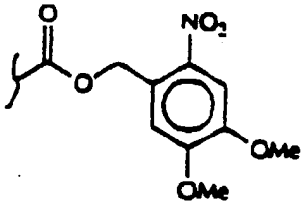
Some preferred photosensitive groups for protecting amino acids and nucleosides during VLSIPS™ have been described in PCT Publication No. WO92/10092. These include nitroveratryloxycarbonyl (NVOC), nitropiperonyloxycarbonyl (NPOC), alpha-methyl-nitroveratryloxycarbonyl (MeNVOC), alpha-methyl-nitropiperonyl (MeNPOC), and the benzylic form of each of these (i.e. NV, NP, MeNV, and MeNP).



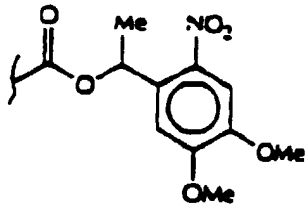
MeNV



MeNP



NVOC



MeNVOC

In addition to VLSIPS™, ortho-nitrobenzyl photolabile protecting groups have been proposed for use in photolithography for electronic device fabrication (see e.g. Reichmanis, et al., J. Polymer Sc. Polymer Chem. Ed. (1985) 23:1-8, incorporated herein by reference for all purposes), and monitoring transport of various molecules and ions in biological milieus (see e.g. Kaplan et al., PNAS USA (1987) 85:6571-6575, incorporated herein by reference for all purposes).

In VLSIPS™, a "basis set" of protected monomers is used to synthesize a diverse collection of polymers. The basis set is, for example, the twenty naturally occurring amino acids having protected alpha-amino groups. It is desirable to have each member of the basis set photolyze at

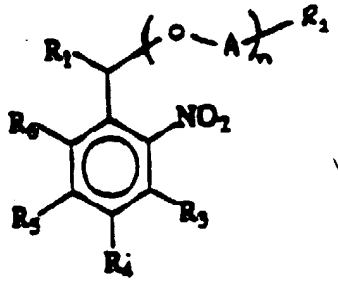
approximately the same rate. Unfortunately, this is often not possible when a single protecting group (e.g. ortho-nitrobenzyloxycarbonyl) is used to protect each member of the basis set. For example, when two different amino acids protected with nitrobenzyloxycarbonyl groups are exposed to radiation of the same wavelength and intensity, they may be deprotected at substantially different rates. Thus, it is desirable to develop methods that "equalize" the photolysis rates of the basis set members.

Although some ortho-nitrobenzyl photoremovable protecting groups have proven useful, additional photoremovable protecting groups having various structures and photolysis rates are desirable.

SUMMARY OF THE INVENTION

The present invention provides new ortho-nitrobenzyl photosensitive protecting groups. These groups can be coupled to amino, carboxyl, hydroxyl, thiol and other moieties of compounds protected during certain chemical reactions. For example, the photosensitive groups of this invention can be used to protect functional groups of nucleosides, amino acids, or saccharides from unwanted side reactions during polymer synthesis.

According to one aspect, the invention provides improved photoremovable protecting groups having the formula:



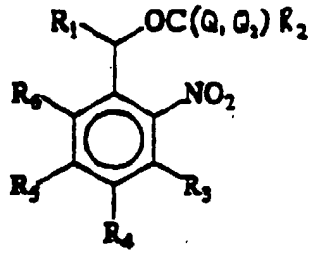
In this structure, n is 0 or 1; A is -C(O)-, -(CQ_1Q_2)-, or -C(S)-; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; and R_2 is a functional group of a molecule such as a natural or unnatural amino acid or peptide, a nucleoside, nucleoside analog, or

oligonucleotide. R_3-R_6 are selected independently from among the groups hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, or a group having the formula -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl. R_3-R_6 may also be a cyclic bridge between adjacent substituents. Exemplary bridges include acetal, ketal, orthoester, thioester, fused aromatic, or ether groups.

In preferred novel embodiments, when n is 0 or A is -C(O)- (i) R_4 and R_5 are not both methoxy when R_1, R_3, and R_6 are hydrogen; (ii) R_1 is not hydrogen, methyl, phenyl, or 2-nitrophenyl when R_3-R_6 are hydrogen simultaneously; and (iii) R_1 is not 2-nitrophenyl or 3,4-dimethoxy-6-nitrophenyl when R_3 and R_6 are hydrogen and R_4 and R_5 are both either hydrogen or methoxy.

The amino acid (and peptide) functional groups protected as described include side chain groups as well as "backbone" groups (i.e. the amine and carboxyl groups that form the peptide bond). Side chain functional groups found on natural amino acids include amines, carboxyl groups, thiols, hydroxy groups, imidazoles, amides, indole groups, etc. The nucleoside, nucleoside analog, and oligonucleotide functional groups include the 2', 3', and 5' ribose hydroxy groups. In addition, the purine and pyrimidine bases of nucleosides can be protected by the above protecting groups. For example, R_2 in the above compound structure can represent base exocyclic amine groups in some embodiments.

A particularly preferred class of photoremovable protecting groups has the formula:



Here, R_2 is a molecule functional group that may be one of the following: an amine, a carboxyl group, a thiol, an imidazole, an amide, a hydroxy group, or the like. Otherwise, R_1, Q_1, Q_2, and R_3-R_6 are the same as defined in the previous structure. In preferred embodiments, the functional group R_2 is found on a natural or unnatural amino acid or peptide, a nucleoside, a nucleoside analog, or an oligonucleotide as described in connection with the above embodiment. Preferred reactive compounds employed to make protected compounds having the above structure include activated ortho-nitrobenzyloxymethyl groups such as ortho-nitrobenzyloxymethyl halides (e.g. ortho-nitrobenzyloxymethyl chlorides). Other active groups that can replace the halo group include hydroxyl, tosyl, mesyl, trifluoromethyl, diazo, azido, and the like.

The invention also includes basis sets of protected natural or unnatural amino or nucleic acids in which a plurality of amino or nucleic acids are protected by photoremovable protecting groups of the invention such that all of the protected amino or nucleic acids are deprotected at substantially the same rate in a particular solvent.

A further understanding of the nature and advantages of the inventions herein may be realized by reference to the remaining portions of the specification and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 schematically illustrates various reaction paths that can be employed to produce alcohols of the ortho-nitrobenzyl protecting groups of this invention;

Fig. 2 schematically illustrates reaction paths to various protected compounds of this invention;

Fig. 3 schematically illustrates reaction paths to various benzyloxycarbonyl protected compounds of this invention;

Fig. 4 schematically illustrates reaction paths to various benzyl protected compounds of this invention; and

Fig. 5 schematically illustrates reaction paths to various benzyloxymethyl protected compounds of this invention.

Fig. 6 presents various compounds for which photolysis data is provided in Table V.

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DESCRIPTION OF THE PREFERRED EMBODIMENTS

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IX. Conclusion

1. Definitions

Certain terms used herein are intended to have the following general definitions

A. Monomer: A member of the set of small molecules which are or can be joined together to form a polymer. Frequently, monomers have at least two different reaction sites (e.g. the amino and carboxyl groups of amino acids). The set of monomers includes but is not restricted to, for example, the set of common L-amino acids, the set of D-amino acids, the set of synthetic and/or natural amino acids, the set of nucleotides and the set of pentoses and hexoses. The particular ordering of monomers within a polymer is referred to herein as the "sequence" of the polymer. The invention is described herein primarily with regard to the preparation of molecules containing sequences of monomers such as amino acids, but could readily be applied in the preparation of other polymers. Such polymers include, for example, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either α-, β-, or ω-amino acids, heteropolymers in which a known drug is covalently bound to any of the above, polynucleotides, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, or other polymers which will be apparent to those of skill in the art. Methods of cyclization and polymer reversal of polymers are disclosed in copending application Serial No. 796,727 (Attorney Docket No. 11509-51), filed on the same date as the present application, entitled "POLYMER REVERSAL ON SOLID SURFACES," incorporated herein by reference for all purposes.

B. Functional Group: A group or moiety on a chemical compound that in some environments imparts certain chemical properties to the compound. More specifically, "functional group" refers to groups that are capable of undergoing reaction such as oxidation or reduction under certain conditions. As used in the context of this invention,

functional group most often refers to a group on a monomer or polymer that can be protected by a photolabile or other group. Typically, functional groups include the reactive sites on a monomer that participate in coupling reactions to produce a polymer. For example, amino acid functional groups include carboxyl and amino groups; nucleic acid functional groups include 2', 3', and 5' hydroxyl groups. Other common functional groups encountered in the context of this invention include side chain groups (thiol, imidazole, indole, etc.) on amino acids; purine/pyrimidine groups (e.g. exocyclic amines) on nucleic acids; amine or carboxyl groups on linkers; and hydroxyl and amine groups on carbohydrates or lipids.

C. Protecting group: A material which is chemically bound to a reactant functional group and which may be removed upon selective exposure to an activator such as electromagnetic radiation. A protecting group prevents the protected functional group from undergoing undesired side reactions. For instance, the amino group of glycine may be coupled to a protecting group to prevent the glycine amino group from reacting during a coupling reaction between the glycine carboxylic terminus and the amino terminus of a growing peptide. Examples of photosensitive protecting groups include nitropiperonyl, nitroveratryl, and nitrobenzyl groups. Other protective groups sensitive to acid or base include t-butyloxycarbonyl or fluorenylmethoxycarbonyl.

D. Basis Set: A set of protected reactants such as monomers used in, for example, a synthesis step in the formation of a polymer array. For example, the 20 naturally occurring amino acids form one basis set and the four naturally occurring deoxyribonucleic acids form another basis set. As a further example, the dimers of the 20 naturally occurring L-amino acids form a basis set of 400 monomers for synthesis of polypeptides. Different basis sets of monomers may be used at successive steps in the synthesis of a polymer. Furthermore, each of the sets may include protected members which are modified after synthesis.

E. Peptide: A polymer in which the constituent monomers are amino acids joined together through amide bonds. Peptides are alternatively referred to as polypeptides. In the context of this specification it should be appreciated that the peptide may include the L-optical or D-optical isomers of α-, β-, or ω-amino acids. In addition, the peptide may include amino acids having unnatural side chains or other deviations from the naturally occurring amino acids. Such "peptides" have been the subject of much study recently, and are discussed by Fauchère, J-L, in Advances in Drug Design, Vol. 15, Testa ed., (1986), which is incorporated herein by reference for all purposes. Peptides are two or more amino acid monomers long, and often more than 20 amino acid monomers long. Standard abbreviations for amino acids are used (e.g., P for proline). These abbreviations are included in Stryer, Biochemistry, Third Ed., 1988, which is incorporated herein by reference for all purposes.

F. Radiation: Energy which may be selectively applied including energy having a wavelength of between 10^{-24} and 10^{4} meters including, for example, electron beam radiation, gamma radiation, x-ray radiation, ultra-violet radiation, visible light, infrared radiation, microwave radiation, and radio waves. "Irradiation" refers to the application of radiation to a material. The photosensitive protecting groups of this invention are typically photolyzed by electromagnetic

radiation in the ultraviolet or visible regions of the spectrum.

Abbreviations: The following frequently used abbreviations are intended to have the following meanings:

BOC: t-butyloxycarbonyl.
BOP: benzotriazol-1-yloxytris-(dimethylamino) phosphonium hexafluorophosphate.
DCC: dicyclohexylcarbodiimide.
DCM: dichloromethane; methylene chloride.
DDZ: dimethoxydimethylbenzyloxy.
DIEA: N,N-diisopropylethylamine.
DMAP: 4-dimethylaminopyridine.
DMF: dimethyl formamide.
DMT: dimethoxytrityl.
DTT: dithiothreitol
FMOC: fluorenylmethyloxycarbonyl.
HBTU: 2-(1H-benzotriazol-1-yl) -1,1,3,3-tetramethyluronium hexafluorophosphate.
HOBT: 1-hydroxybenzotriazole.
NBOC: 2-nitrobenzyloxycarbonyl.
NMP: N-methylpyrrolidone.
NPOC: 6-nitropiperonyloxycarbonyl.
NV: 6-nitroveratryl.
NVOC: 6-nitroveratryloxycarbonyl.
TFA: trifluoracetic acid.
THF: tetrahydrofuran.

II. Function of Protecting Groups

Protecting groups of the present invention can be used in conjunction with solid phase oligomer syntheses, such as peptide syntheses using natural or unnatural amino acids, nucleotide syntheses using deoxyribonucleic and ribonucleic acids, oligosaccharide syntheses, and the like. In a particularly preferred embodiment, the protecting groups of this invention are used to synthesize an array of polymers according to a VLSIPS™ process. Such processes employ a substrate containing oligomers or other materials protected

with photosensitive groups. Selected regions of the substrate are irradiated to create regions having reactive moieties that are essentially free of side products resulting from the protecting group.

The protecting groups serve two purposes: (1) protecting the substrate surface from unwanted reactions, and (2) blocking a reactive functional group of the monomer to prevent self-polymerization or other reaction. For instance, attachment of a protecting group to the amino terminus of an activated amino acid, such as an N-hydroxysuccinimide-activated ester of the amino acid, prevents the amino terminus of one monomer from reacting with the activated ester portion of another during peptide synthesis. Alternatively, the protecting group may be attached to the carboxyl group of an amino acid to prevent reaction at this site. Most protecting groups can be attached to either the amino or the carboxyl group of an amino acid, and the nature of the chemical synthesis will dictate which reactive groups will require a protecting group. Analogously, attachment of a protecting group to the 5'-hydroxyl group of a nucleoside or nucleoside derivative during oligonucleotide synthesis using for example, phosphoramidite coupling chemistry, prevents the 5'-hydroxyl of one nucleoside from reacting with the 3'-activated phosphate-triester of another. In other embodiments, the photolabile protecting group may be attached to the 2'- or 3'-hydroxyl group of a nucleoside.

In alternative embodiments, the photosensitive protecting groups of this invention can be employed to protect side chain functionalities of amino acids during peptide syntheses employing conventional acid or base labile protecting groups such as BOC (t-butyloxycarbonyl) or FMOC (fluorenylmethoxycarbonyl) on the alpha backbone amine. In some preferred embodiments, the photolabile protecting groups protect any one or a combination of the following groups: the thiol group of cysteine; the basic residues (amine and imidazole groups) of lysine, arginine, and histidine; the acidic carboxy groups of aspartic and glutamic acids; the hydroxy groups of serine, threonine, and tyrosine; and the

heterocyclic indole ring of tryptophan. Of course, other amino acid side chains such as those occurring on non-natural amino acids can likewise be protected by the photosensitive groups of the present invention. Further, the purine and pyrimidine bases (and particularly the exocyclic amine groups) of nucleoside and nucleoside derivatives likewise can be protected with the photosensitive groups of this invention.

Regardless of the specific type of compound being synthesized, protecting groups are employed to protect a moiety on a molecule from reacting with another reagent. Typically, the reaction to be blocked will be the formation of a covalent bond such as a peptide bond between two amine acid molecules. However, the protecting groups of this invention can also block non-covalent reactions. For example, the photosensitive protecting groups of this invention can be used to "cage" (i.e. provide protection based upon steric hindrance) biotin or a biotin analog, thus preventing binding with avidin. Useful applications of this strategy are described in Barrett et al., PCT Publication No. WO 91/07087, incorporated herein by reference for all purposes.

Protecting groups of the present invention preferably have the following general characteristics: they prevent selected reagents from modifying the group to which they are attached; they are stable (that is, they remain attached to the molecule) to the synthesis reaction conditions; they are removable under conditions that do not adversely affect the structure to which they are attached; and once removed, they do not react appreciably with the surface or surface-bound oligomer. In some embodiments, liberated byproducts of the photolysis reaction can be rendered unreactive toward the growing oligomer by adding a reagent that specifically reacts with the byproduct. The selection of a suitable protecting group will depend, of course, on the chemical nature of the monomer unit and oligomer, as well as the specific reagents they are to protect against.

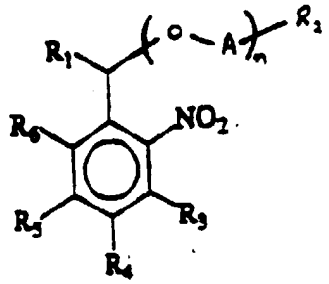
The removal rate of the protecting groups depends on the wavelength and intensity of the incident radiation, as well as the physical and chemical properties of the protecting

group itself. Preferred protecting groups are removed at a faster rate and with a lower intensity of radiation. For example, at a given set of conditions, MeNVOC and MeNPOC are photolytically removed from the N-terminus of a peptide chain faster than their counterparts, NVOC and NPOC. Therefore for most applications, MeNVOC and MeNPOC are preferred over NVOC and NPOC.

In many embodiments, the photosensitive protecting groups will be removable by radiation in the ultraviolet (UV) or visible portion of the electromagnetic spectrum. In preferred embodiments, the photosensitive protecting groups are sensitive to an electromagnetic spectral region that does not significantly overlap the absorption band of the monomers or polymers being protected. Higher energy radiation (i.e. low wavelength radiation) is generally more likely to damage monomers such as amino acids and nucleosides. For example, the four naturally occurring bases in deoxynucleosides absorb 250-280 nm light very strongly. In addition, short wave ultra-violet light is known to photo-destruct DNA formation of thymine dimers. Thus, in most preferred embodiments, the protecting groups will be removable by radiation in the near UV or visible portion of the spectrum. Of course, each collection of monomers (basis sets) will have its own characteristic absorption bands which can be avoided by choosing protecting groups that are sensitive to wavelengths well removed from the absorption band.

III. Preferred Ortho-nitrobenzyl Protecting Groups

Preferred photochemical protecting groups of the present invention have the general formula:



In this structure, n is 0 or 1; A is -C(O)-, -(CQ_1Q_2)-, or -C(S)-; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; and R_2 is a functional group of a molecule such as a natural or unnatural amino acid or peptide, a nucleoside, nucleoside analog, or oligonucleotide. R_3-R_6 are selected independently from among the groups hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy (e.g., CH_3 C(O)O-) nitro, alkylthio, arylthio, hydroxyl, halogen, or a group having the formula -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl. R_3-R_6 may also be a cyclic bridge between adjacent substituents. Exemplary bridges include acetal, ketal, orthoester, thioester, fused aromatic (which together with the first benzene ring forms a derivative of naphthalene, quinoline, anthracene, etc.), or ether groups.

In preferred novel embodiments, when n is 0 or A is -C(O)- (i) R_4 and R_5 are not both methoxy when R_1, R_3, and R_6 are hydrogen; and (ii) R_1 is not hydrogen, methyl, phenyl, or 2-nitrophenyl when R_3-R_6 are hydrogen simultaneously. Further, compounds having hydroxyl substituents on the phenyl ring, and/or alkoxy or aryloxy substituents on the benzyl carbon may be too reactive for certain applications, and therefore are generally less preferred.

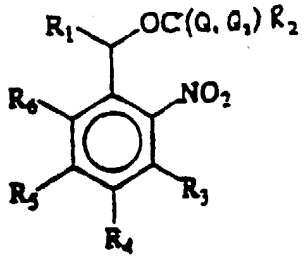
The amino acid (and peptide) functional groups protected as described include side chain groups as well as "backbone" groups (i.e. the amine and carboxyl groups that form the peptide bond). Those of skill in the art will recognize that a variety of other functional groups can be protected by the above photosensitive groups. Specific examples include hydroxy and amino groups on saccharides, hydroxy groups on steroids, and amino groups attached to linkers on a substrate surface (see PCT Publication No. WO92/10092, previously incorporated by reference). In addition, certain molecules can be "caged" to provide steric protection against binding. For example, biotin and biotin analogs can be caged with the above photosensitive groups to

prevent binding with avidin. The protecting group can be coupled through the biotin urea groups.

Although carboxyl, amino, and hydroxy groups of amino acids and nucleosides can, in principle, be protected with either benzyl or benzyloxycarbonyl protecting groups (i.e. n=0 and 1, and ignoring the possibility that A is -CH2- or -C(S)-), certain couplings are preferred. Specifically, amino acid carboxyl groups preferably are protected with benzyl form photosensitive groups (n=0), while amino acid amine groups and nucleoside hydroxy groups preferably are protected with benzyoxycarbonyl form of the photosensitive groups (n=1).

When a primary amine group (e.g. those found on most natural amino acids) is protected by a benzylic group (n=0) of the above structure, the amine nitrogen must be further protected for some applications. For example if the amine is not further protected during peptide synthesis using VLSIPS™, the free amine hydrogen may react with other amino acids activated with coupling reagents such as BOP. If, however, a second protecting is employed, no free hydrogen atoms are present on the amine to act as a reaction sites. Preferably, the additional protection is provided by C_1-C_6 alkyl, aryl, acyl, or benzyl groups. More preferably, the additional protective group is methyl or acetyl. Such compounds are readily synthesized by methods well-known in the chemical arts. For example, following the coupling of the above-described photoprotective group to an amino acid (made, e.g., by using an activated benzyl derivative of the photoprotective group), the photoprotected amino acid may be reacted with a reagent known to methylate amino acid nitrogen moieties, such as methyl iodide, to form the desired protected amino acid.

A particularly preferred class of protecting groups is the 6-nitrobenzyloxymethyl groups which have exhibited very rapid photolysis rates and have the general formula:

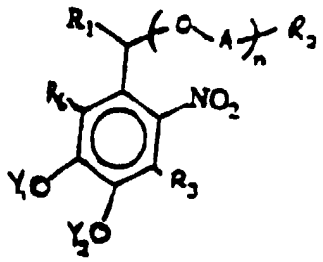


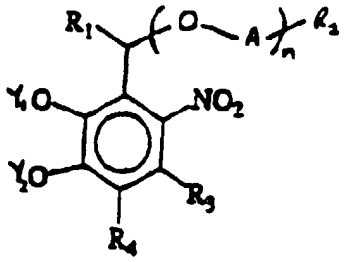
Here, R_2 is a molecule functional group that may be one of the following: an amine, a carboxyl group, a thiol, an imidazole, an amide, a hydroxy group, and the like. Otherwise, R_1, Q_1, Q_2 and R_3-R_6 are the same as defined in the above structure. In preferred embodiments, the functional group R_2 is found on a natural or unnatural amino acid or peptide, a nucleoside, a nucleoside analog, or an oligonucleotide. Both side chain and backbone groups may be protected. As in the above general embodiment, the photosensitive groups can also be used to protect functional groups found on a variety of compounds such as carbohydrates, lipids, biotin analogs, linker molecules, etc.

Preferred reactive compounds employed to make protected compounds having the above structure include activated ortho-nitrobenzyloxymethyl compounds such as ortho-nitrobenzyloxymethyl halides (e.g. ortho-nitrobenzyloxymethyl chlorides). Other active groups that can replace the halo group include hydroxyl, tosyl, mesyl, trifluoromethyl, diazo, azido, and the like.

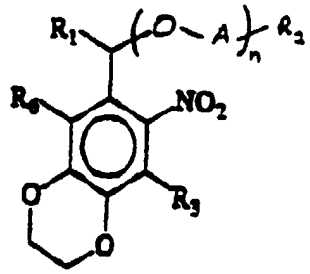
Another class of preferred protecting groups includes compounds in which R_5 and R_6 are both alkyloxy groups or together form a cyclic bridge acetal or ketal. In particularly preferred embodiments R_5 is hydrogen or methyl and R_3 and R_4 are hydrogen. In alternative preferred embodiments, R_4 and R_5 are alkoxy groups or together form the cyclic acetal or ketal. Preferably, R_3 and R_6 will then be hydrogen. Such compounds are made by standard methods such as those described in Greene, Protective Groups in Organic Synthesis, Wiley 1981, which is incorporated herein by reference for all purposes. One example is where R_4 and R_5 or R_5 and R_6 form a methylene acetal or an acetonide: (-O-CH_2-O-

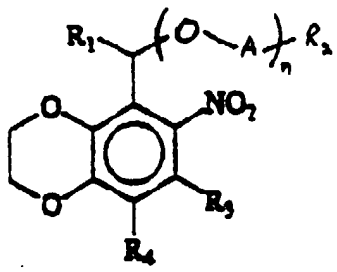
, or $-O-C(CH_3)_2-O-$). In the structures below, Y_1 and Y_2 are independently C_1-C_8 alkyl groups, or fused to form a cyclic bridge acetal or ketal.



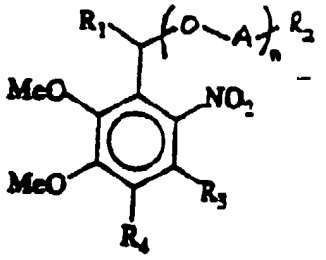


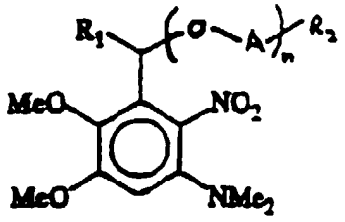
In addition to acetals and ketals, the photoprotecting groups of the invention may also include compounds where adjacent benzene ring substituents form a ring having the formula $-O-CRR'-O-$ or $-O-CRR'-CR''R'''-O-$, where R, R', R", and R‴ can independently be hydrogen, C_1-C_8 alkyl, aryl, benzyl, alkoxy, aryloxy, carboxy, alkyl carboxylic acid ester (i.e. $-C(O)O$-alkyl), or carbonyl. Specific examples include cyclic orthoesters ($-O-CRR'-O-$, where R and R' are alkoxy, aryloxy, or carboxy) or cyclic ethers ($-O-CRR'-CR''R'''-O-$ where R, R', R", and R‴ are selected from the group hydrogen, alkyl, aryl, benzyl, alkoxy, aryloxy, or carboxy). In addition, R and R' together or R" and R‴ together may be a carbonyl oxygen (e.g. forming the following bridges: $-O-C(O)CH_2-O-$ or $-O-CH_2C(O)-O-$). These compounds are readily synthesized by known methods (see, e.g., Greene). A preferred cyclic ether has R, R', R", and R‴ hydrogen (i.e., $-O-CH_2-CH_2-O-$). Preferred substitution patterns are those at the R_2 and R_5, and R_5 and R_6 positions:



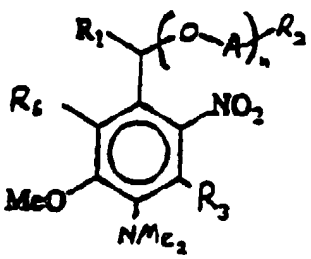


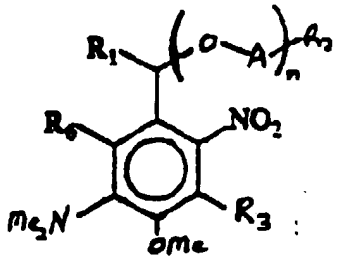
Still other preferred compounds are those where R_5 and R_6 are methoxy. In a specific preferred embodiment R_3 is dimethylamino and R_4 is hydrogen:



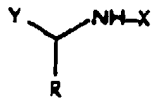


Yet still other preferred compounds are formed when R_4 is methoxy and R_5 is dimethylamino, or, conversely, where R_4 is dimethylamino and R_5 is methoxy:





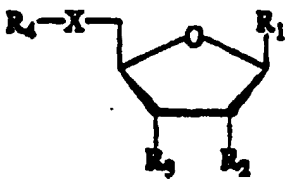
As noted, the photoremovable protecting groups of the present invention can be attached to an activated ester of a natural or unnatural amino acid or peptide at the amino terminus. For use in VLSIPS™ or other peptide synthesis techniques, an amine protected amino acid may have the following structure:



where R is the side chain of a natural or unnatural amino acid, X is a photoremovable protecting group according to this invention, and Y is an activated carboxylic acid derivative. The activated ester, Y, is preferably a reactive derivative having a high coupling efficiency, such as an acyl halide, mixed anhydride, N-hydroxysuccinimide ester, perfluorophenyl ester, or urethane protected acid, and the like. Other activated esters and reaction conditions are well known (See

Atherton et al., "Solid Phase Peptide Synthesis" 1989, IRL Press, London, incorporated herein by reference for all purposes).

As noted, the photoreactive protecting groups of the present invention are useful for protecting both natural and unnatural nucleosides or nucleotides. More specifically, the 2', 3', and/or 5' hydroxyl functions of such compounds. Such compounds can therefore have the general structure:

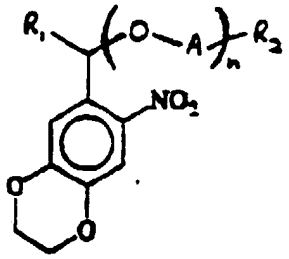


X may be oxygen or sulphur. R_1 is a purine or pyrimidine base or analog thereof (for example, adenine, cytosine, thymine, guanine, uracil, 6-ketoadenine, or an analog thereof). R_2-R_4 may be one of the commonly used protecting groups (see below), or a photoreactive group as described above. Typically, one position will be protected with a photoreactive protecting group while the other position(s) will be (a) protected or substituted with one of the commonly used protecting groups, or (b) activated with one of the commonly reactive groups. However, more than one position may be protected with a photoreactive protecting group of the invention.

With respect to R_2, R_3, and R_4, these may be hydrogen, hydroxy, an oligonucleotide or one of the commonly used protecting groups: alkoxy, tetrahydropyranyl, β-benzoylpropionyl, acetyl, or silyl. They can also be -NRR', -OP(O)(OR")(OR"'), $-OP(O)O_2^{-2}$, $-OP(O)O_2H^-$, -OP(O)(OR")(NRR'), or -OP(OR")(NRR') (i.e. phosphoramidite) where R and R' are independently selected from the group consisting of C_1-C_3 alkyl, aryl, benzyl, or acetyl, and R" and R"' are selected independently from the group consisting of C_1-C_3 alkyl, aryl, benzyl, or C_1-C_3 cyanoalkyl. R_2 or R_3 also together form a

cyclic acetal, ketal, orthoester, or ether. In some preferred embodiments, R_4 can be triphenylmethyl, di-p-methoxytrityl, dimethylpropanoyl. These compounds and their methods of synthesis are well-known in the art (see, e.g., Fuhrhop and Penzlin, Organic Synthesis Concepts, Methods, and Starting Materials, Verlag Chemie 1983, or Gait, Oligonucleotide Synthesis a Practical Approach, IRL Press 1984, both of which are incorporated herein by reference for all purposes).

An especially preferred photoreactive protecting group for use with nucleosides and nucleotides has the formula:



IV. Choice of Sidechain Protecting Groups

It will be appreciated by those skilled in the art that functional groups other than those directly involved in coupling during polymer synthesis may have to be protected. It will also be appreciated that such protecting groups should not be removable under coupling conditions. Thus, for example, a desired polypeptide will not be contaminated as a result of cross reactions between sidechains which have inadvertently become unprotected and other monomers or the sidechains of other amino acids. Since the removal of the photoreactive protecting groups of the present invention typically creates an acidic environment, care must be taken to choose sidechain protecting groups which are moderately acid stable.

Herein, the term "acid stable" means that the sidechain protecting group in question is not removed in appreciable amounts under conditions where the effective pH is less than about 7. Determining stability will depend on the particular amino acid sidechain as well as the sidechain protecting group which is chosen. For lysine, it has been

found that ε-amino moieties protected with BOC (t-butyloxycarbonyl) tend to degrade over time in 5mM sulfuric acid. Thus, a protecting group for the ε-amino group of this amino acid should be less acid labile than BOC. Generally, trityl protecting groups are also too sensitive to protect ε-amino groups. One preferred protecting group for the ε-amino acid of lysine is dimethoxybenzyloxycarbonyl. Another preferred protecting group is FMOC (9-fluorenylmethyloxycarbonyl). These groups are well-known in the art as are the techniques for forming the appropriately protected lysine amino groups.

Although trityl is not preferred with respect to lysine, trityl is stable enough to be used with cystine, asparagine, and glutamine. Generally, however, asparagine and glutamine do not require protection. With respect to histidine, protection of the imidazole nitrogen is needed during addition to the polymer in order to prevent self-condensation and racemization. However, once the histidine is bound to the substrate, protection is no longer required. FMOC and trityl are examples of suitable protecting groups for the imidazole nitrogen of histidine. It will be appreciated by those of skill in the art that various other protecting groups may be used depending upon the application.

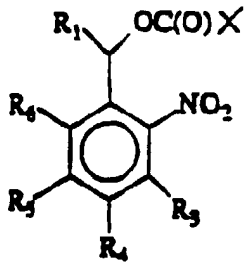
V. Synthesis of Protected Compounds

The protected compounds of the present invention often can be prepared via a process employing a benzyl alcohol of the protecting group. Various synthetic routes to some useful benzyl alcohols are shown in Fig. 1. In the displayed benzyl alcohols, R, R', and R" represent hydrogen, methyl, C_2 to C_9 alkyl, and other groups in accordance with the structures presented above. Each arrow shown in Fig. 1 depicts a different reaction pathway, employing different reagents. Typical reagents for suitable use in the various reaction paths are as follows: (a) RC(O)Cl and aluminum chloride; (b) DMF and $POCl_3$; (c) R'I or R"I or $MeBr_2$ or $EtBr_2$ and potassium carbonate; (d) concentrated nitric acid or other nitration agent; (e) $NaBH_4$; and (f) RMgBr or RLi.

Fig. 2 displays reaction paths for making various protected compounds from benzyl alcohols. In the structures shown, X denotes substitution on the phenyl ring, R denotes substitution on the benzylic hydrogen, and Z denotes an oxygen or sulfur atom. In reaction path (a), the alcohol is reacted with ClC(Z)OR' and triethylamine or ClC(Z)SR' and triethylamine to form the protected thiol or alcohol shown. The reactants (other than the benzyl alcohol of the protecting group) and the products for the various other reactions are as follows: (b) reaction with $ClCH_2OR$ and triethylamine produces a protected alcohol; (c) reaction with Z=C=NR' or ClC(Z)NR'R" produces a protected amine; (d) reaction with $ClCH_2SR'$ and triethylamine produces a protected thiol; and (e) reaction with R'C(O)OH, DCC, and DMAP or with R'C(O)Cl and triethylamine produces a protected carboxylic acid.

Fig. 3 schematically depicts some general reactions that can be employed to produce benzyloxycarbonyl (and thiocarbonyl) protected functional groups. A shown, a benzyl alcohol is reacted through a chloroformate intermediate to produce the desired compounds. In Fig. 3, X denotes substitution on the phenyl ring, R denotes substitution on the benzylic carbon, and Z denotes an oxygen or sulfur atom. Typical reagents employed in the noted routes are as follows: (a) $C(O)Cl_2$ or $C(S)Cl_2$; (b) R'OH and triethyl amine; (c) R'SH and triethyl amine; (d) R'R"NH; and (e) $H_2NC(O)R'$

In one specific embodiment, the appropriate benzyl alcohol can be reacted with phosgene or other agent to produce an activated benzyloxycarbonyl derivative of the protecting group. These compounds are then used to produce the above-described benzyloxycarbonyl-protected groups (e.g. n is 1 and A is -C(O)- in the above generic structures). These groups are preferably coupled to the amino nitrogen of a natural or unnatural amino acid; or the 2', 3', or 5' oxygen of a natural or unnatural nucleoside or nucleotide using standard methods, for example, reacting the amino group of an amino acid or a ribose hydroxyl moiety of a nucleoside with the desired activated benzyloxycarbonyl derivative. Examples of such activated protecting groups have the general formula:



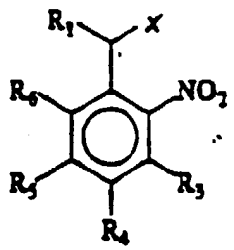
where X is halogen, mixed anhydride, phenoxy, p-nitrophenoxy, N-hydroxysuccinimide, hydroxyl, tosyl, mesyl, trifluoromethyl, diazo, azido, and the like. R_1 is hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy. R_3-R_6 are selected independently from among the groups hydrogen, alkoxy, aryloxy, benzyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, or a group having the formula -NR'R" where R' and R" are selected independently from the group consisting of C_1-C_8 alkyl, aryl, or benzyl. R_3-R_6 may also be a cyclic bridge between adjacent substituents. Exemplary bridges include acetal, ketal, orthoester, thioester, phenyl, or ether groups. Compounds falling within this formula are readily formed using known standard techniques such as those described in March, Advanced Organic Chemistry, 3rd ed., Wiley 1985, or Carey and Sundberg, Advanced Organic Chemistry Part B: Reactions and Synthesis, 2nd ed., Plenum 1984, both of which are incorporated herein by reference for all purposes. It should be noted that the corresponding benzyloxythiocarbonyl (employing the -C(S)- group in place of -C(O)-) can be prepared by analogous methods.

Fig. 4 schematically depicts various reaction pathways from a benzyl alcohol starting material to benzyl-protected compounds via an activated benzyl intermediate. In the compounds shown, X denotes substitution on the phenyl ring and R denotes substitution on the benzylic carbon. Typical reagents used in the various reaction steps are as follows: (a) $SOCl_2$; (b) R'OH and triethylamine; (c) R'SH and triethylamine; (d) R"R'NH; and (e) $H_2NC(O)R'$ and triethylamine; (f) R'C(O)OH and triethylamine.

In a specific embodiment, the carboxy terminus of an amino acid protected with a benzylic photoactivatable group

can be formed by esterifying the carboxy group with an activated benzyl derivative of the protecting group. Likewise, the amino terminus of an amino acid or a hydroxy group of a nucleoside (or nucleoside derivative) protected with a benzylic group can be formed by alkylating the amino or hydroxy group with an activated benzyl derivative of the protecting group. As noted above, benzyoxycarbonyl groups are more preferred for protecting amine groups of amino acids and hydroxy groups of nucleosides. Further, if a benzylic form of a photoreactive protecting group is used to protect the amino nitrogen of an amino acid, that nitrogen may have to be further protected with an alkyl, aryl, benzyl, or acyl group.

Examples of activated benzyl derivatives have the general formula:

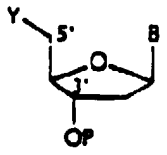


where X is halogen, hydroxyl, tosyl, mesyl, trifluoromethyl, diazo, azido, and the like. R_1 is hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy. R_2-R_6 are selected independently from among the groups hydrogen, alkoxy, aryloxy, benzyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, or a group having the formula -NR'R" where R' and R" are selected independently from the group consisting of C_1-C_8 alkyl, aryl, or benzyl. R_3-R_6 may also be a cyclic bridge between adjacent substituents. Exemplary bridges include acetal, ketal, orthoester, thioester, phenyl, or ether groups.

Another method for generating compounds protected with benzylic protecting groups is to react the benzylic alcohol derivative of the protecting group with an activated ester of the compound to be protected. For example, to protect the carboxyl terminus of an amino acid, an activated ester of the amino acid is reacted with the alcohol derivative of the protecting group. Examples of activated esters

suitable for such uses include halo-formate, mixed anhydride, imidazolyl formate, acyl halide, and also include formation of the activated ester in situ the use of common reagents such as DCC and the like. See Atherton et al. (previously incorporated by reference) for other examples of activated esters.

To protect the 5'-hydroxyl group of a nucleic acid with a benzylic protecting group, a derivative having a 5'-activated carbon is reacted with the alcohol derivative of the protecting group. Examples of nucleotides having activating groups attached to the 5'-hydroxyl group have the general formula:

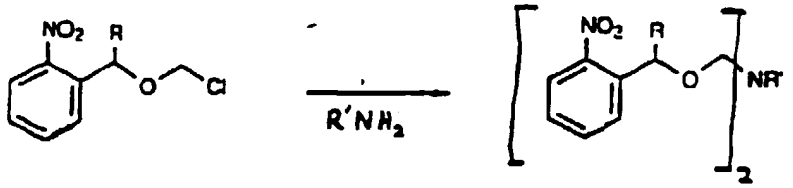


where Y is a halogen atom, a tosyl, mesyl, trifluoromethyl, azido, or diazo group, and the like. Similarly, the 2'- and 3'- hydroxyl moieties of nucleotide, nucleosides and derivatives thereof may be protected.

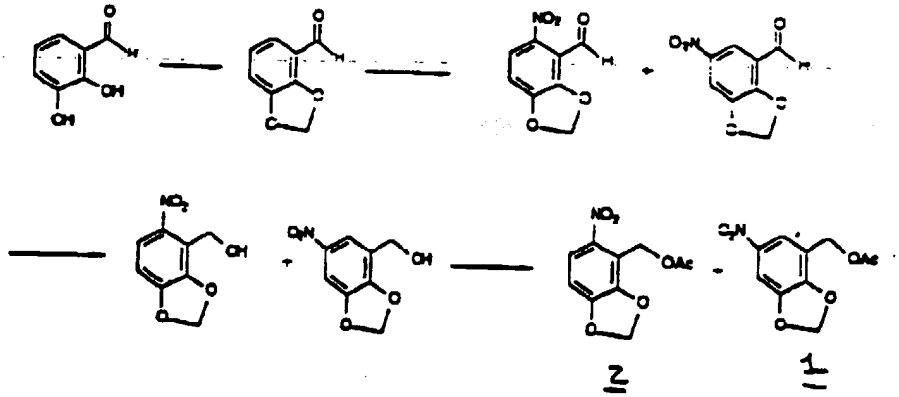
Benzyloxymethyl-protected compounds can be synthesized using standard techniques known in the chemical arts. For example, 6-nitroveratryloxymethyl chloride (NVOMCl) is readily made from 6-nitroveratryl alcohol upon exposure to gaseous HCl and paraformaldehyde following the methods described in Organic Syntheses, Vol. 6, pp. 101-103 (1988), which is incorporated herein by reference. Reaction of NVOMCl with the 5'-hydroxyl group of a nucleoside to form the protected 5'-NVOM nucleoside is readily performed using techniques such as those described in Greene, or by Corey, et al., in Tetrahedron Letters, No. 11, pp. 809-812 (1976), which is incorporated herein by reference. For example, the hydroxy group to be protected is reacted with the activated form of the benzyloxymethyl protecting group in the presence of a tertiary amine to form the protected compound, a benzyloxymethylether. Primary and secondary amines as well as carboxylic acids can protected in an analogous manner.

Examples of reactions to produce benzyloxymethyl-protected compounds via a chloromethyl ether intermediate are schematically presented in Fig. 5. As in Figs. 2 to 4, X denotes substitution on the phenyl ring and R denotes substitution on the benzylic carbon. Typical reagents that can be used in the various reaction steps are as follows: (a) CH_2O and HCl; (b) R'OH and triethyl amine; (c) R'SH and triethylamine; (d) R"R'NH; (e) $H_2NC(O)R'$ and triethylamine; and (f) R'C(O)OH and triethylamine.

If excess activated benzyloxymethyl compound is reacted with a primary amine to be protected, an amine having two protecting groups will be formed. Such protected compounds require two photons to be completely deprotected.



Example 1



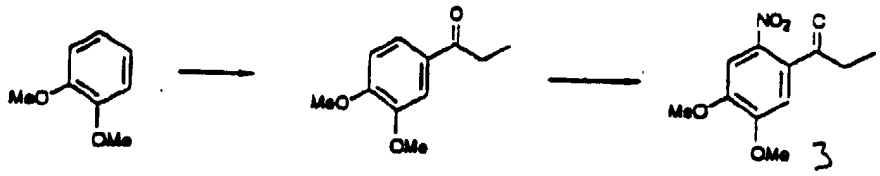
A solution of 2,3-dihydroxy benzaldehyde (1.38 g, 0.01 mol), KF (2.90 g, 0.05 mol), and CH_2Br_2 (1.91 g, 0.011 mol) in 30 mL of DMF was heated to 110-120°C for 2 hours. The reaction mixture was cooled to room temperature and partitioned between ether and water. The organic phase was washed (sat. NaCl), dried ($MgSO_4$), and evaporated to give 1.46 g of a light brown oil. Chromatography on silica gel with 10%

EtOAc in hexanes afforded 1.14 g of 2,3-methylenedioxybenzaldehyde as a light yellow solid.

The benzaldehyde compound was dissolved in 40 mL of 70% HNO_3 cooled to 0°C. After stirring for 30 min., the reaction mixture was poured into cold water and extracted with EtOAc. The organic phase was washed (sat $NaHCO_3$), dried ($MgSO_4$), and evaporated to give 1.2 g of a yellow solid which was used without further purification. The solid was taken up in 25 mL of anhydrous THF and treated with $NaBH_4$ (466 mg, 0.12 mol) at room temperature for 1 hour. The reaction mixture was partitioned between sat. NH_4Cl and EtOAc. The organic phase was dried ($MgSO_4$) and evaporated to afford 1.18 g of the benzyl alcohol as a light yellow solid, which was used without further purification.

The alcohol was dissolved in 20 mL of CH_2Cl_2 and treated with DMAP (0.26 g, 0.0018 mol) and Ac_2O (1.5 g, 0.15 mmol) for 18 hours. The reaction mixture was partitioned between CH_2Cl_2 and 3 N HCl. The organic phase was washed (sat. $NaHCO_3$), dried ($MgSO_4$), and was evaporated to give 1.4 g of a mixture of the two regioisomers as a light yellow solid. Chromatography on silica gel with 10% hexanes in CH_2Cl_2 afforded 0.15 g of the less polar compound 1 as a light yellow solid and 0.40 g of the more polar compound 2 as a light yellow solid and 0.69 g of a mixture of the two.

Example 2

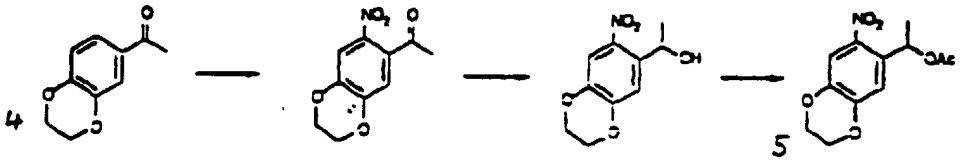


A solution of veratrole (2.00 g; 14.5 mmol) and propionyl chloride (1.4 mL; 16.1 mmol) in 15 mL of CS_2 cooled to 0°C was treated with $AlCl_3$ (2.00 g; 15.0 mmol). After stirring for 30 min., the mixture was allowed to warm to room temperature and turned deep red in color. The reaction

mixture was quenched after an additional 4.5 hours by cooling to 0°C and slowly adding water. The residue was partitioned between EtOAc and 1 N HCl, and the organic phase washed (sat. NaCl), dried ($MgSO_4$), and concentrated to yield 3.00 g of a yellow solid. The crude ketone was used without further purification.

The crude ketone was dissolved in 15 mL of glacial acetic acid and added to 100 mL of 70% HNO_3 cooled to 0°C. The reaction mixture darkened in color within 5 min. After stirring for 20 min., the mixture was allowed to warm to room temperature for an additional 1 hour. The mixture was poured into cold water, and was extracted with EtOAc. The organic phase was washed (sat,. NaCl), dried ($MgSO_4$), and evaporated to give a yellow solid. Recrystalization from MeOH/H_2O afforded 1.716 g (50% yield for two steps) of 3 as tan crystals.

Example 3



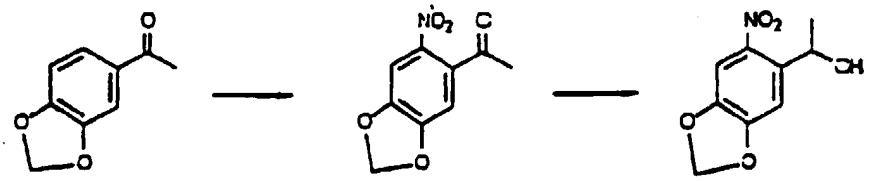
1,4-Benzodioxan-6-yl methyl ketone (1.00 g; 5.61 mmol) was dissolved in 25 mL of 70% HNO_3 cooled to 0°C. The reaction mixture darkened in color within 5 min. The solution was warmed to room temperature after 5 min and was quenched /after an additional 2.5 hours by pouring into cold water and was extracted with EtOAc. The organic phase was washed (sat. NaCl, sat $NaHCO_3$), dried ($MgSO_4$), and evaporated to give a tan solid which was used without further purification.

The crude nitrophenyl compound was dissolved in 25 mL of anhydrous THF and treated with $NaBH_4$ (400 mg; 10.6 mmol) at room temperature. The solution darkened to dark brown after 2 min. After stirring for 30 min., 10 mL of EtOH was added and the stirring continued for an additional 30 min. The solution was partitioned between EtOAc and sat. NH_4Cl, and

the organic phase was dried ($MgSO_4$), and evaporated to afford a brown oil.

The crude benzyl alcohol was taken up in 20 mL of CH_2Cl_2 and 6 mL of pyridine and was treated with 2 mL of Ac_2O and 10 mg of DMAP for 14 hours. The reaction mixture was partitioned between EtOAc and 1 N HCl, and the organic phase was dried ($MgSO_4$), and concentrated to give a brown oil. Chromatography on silica gel with 100% CH_2Cl_2 gave 0.68 g of a yellow oil, which was shown to be a mixture of three compounds by NMR analysis. Chromatography of this mixture on silica gel with 25% EtOAc in hexanes afforded 255 mg of pure 5 as a light yellow solid, MP 82-85°C, and an additional 238 mg of slightly impure 5 as a light yellow solid.

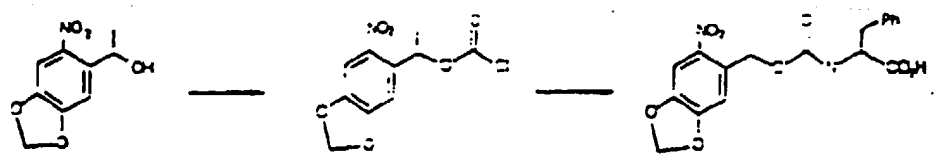
Example 4



Piperonal (21.65 g; 129 mmol) was slowly added to 175 mL of 70% HNO_3 cooled to 10°C. The dark reaction mixture was allowed to warm to room temperature and stirred for 1 hour total. The resultant slurry was poured into cold water and filtered to give a light yellow solid. Recrystalization from $MeOH/H_2O$ afforded 21.46 g of light yellow crystals having a melting point of 108-112°C (80% yield).

To a solution of the nitroacetophenone (14.7 g; 70.4 mmol) in 500 mL of MeOH and 100 mL of $CHCl_3$ was added $NaBH_4$ (2.71 g; 71.6 mmol). After stirring for 5 hours, the solution was partitioned between $CHCl_3$ and sat. NH_4Cl. The organic phase was dried ($MgSO_4$), and evaporated to give a yellow solid. Recrystalization from $MeOH/H_2O$ afforded 14.24 g of the alcohol as light yellow crystals (96% yield).

A solution of the nitropiperonyl alcohol (1.00 g; 4.74 mmol) in 25 mL of THF was treated with phosgene (8 mL of



1.93 M in toluene; 15.4 mmol) at room temperature for 12 hours. The excess phosgene and solvent were removed under reduced pressure to give a tan solid, which was used without any further purification.

A solution of the crude chloroformate in 10 mL of dioxane was added to a solution of L-phenylalanine (540 mg; 3.27 mmol) and $NaHCO_3$ (680 mg; 8.09 mmol) in 15 mL of H_2O and 5 mL of dioxane. The reaction mixture was a light brown, homogeneous solution. The solution was stirred at room temperature for 26 hours and then was partitioned between ether and 0.1 N NaOH. The ether phase was discarded and the aqueous phase was acidified to pH 2 with 3 N HCl and was extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$) and evaporated to give a white solid. Chromatography on silica gel with 15% MeOH in $CHCl_3$ afforded 1.11 g (84% yield) of MeNPOC-L-Phe-OH as a white solid, MP 144-154°C (decomposes).

Example 5

Alpha-methyl-6-nitropiperonyl alcohol was prepared as follows. All anhydrous reagents, methylene chloride, pyridine, triethylamine, and diisopropylethyl amine were obtained from Aldrich Chem Co. N-4-isobutyryl-2'-deoxycytidine, N6-phenoxyacetyl-2'-deoxyadenosine, N2-phenoxyacetyl-2'-deoxyguanosine, 2'-deoxythymidine, and 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite were purchased from Sigma Chem Co. 4,5-methylenedioxy-2-nitroacetophenone was purchased from Lancaster Synthesis and 1.93M phosgene in toluene was from Fluke Chem Co.

^1H NMR spectra were measured on a Varian Gemini 300 MHz Spectrometer and are reported in ppm. Mass Spectra were obtained from the U. C. Berkeley Mass Spectrometry Laboratory.

4,5-methylenedioxy-2-nitroacetophenone (30 g, 0.144 mol) was dissolved in 200ml ethanol and 10ml of tetrahydrofuran. Sodium borohydride (5.5 g, 0.144 mol) was

added to the stirred suspension over a 5 min. period. The suspension was stirred for an additional hour at room temperature, poured into 1.2L of ice water, and acidified to pH 2 with 1N HCl. The yellow solid was collected by filtration, washed with water, and dried *in vacuo*. The solid was recrystallized from 1:1 toluene/hexane to yield 22.8 g (75%) of α-methyl-6-nitropiperonyl alcohol. ^{1}H NMR ($CDCl_3$): 1.55 (d, 3H) CH_3; 5.45 (q, 1H) CH; 6.1 (m, 2H) O-CH_2-O; 7.3 and 7.5 (s, 2H) aromatic. MS

Alpha-methyl-6-nitropiperonyloxycarbonyl chloride was prepared as follows. Phosgene (500ml, 1.93M in toluene) was added to a rapidly stirring solution of (20 g, 0.095 mol) in 700ml anhydrous tetrahydrofuran . After stirring overnight at room temperature the mixture was evaporated to dryness *in vacuo* resulting in a thick brown oil. Tituration with hexane yielded 20 g (73%) of α-methyl-6-nitropiperonyloxycarbonyl chloride as a yellow-brown solid. ^{1}H NMR ($CDCl_3$): 1.7 (d, 3H) CH_3; 6.15 (m, 2H) O-CH_2-O; 6.5 (q, 1H) CH; 7.05 and 7.55 (s, 2H) aromatic.

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-2'-deoxynucleosides was prepared as follows. N-protected deoxynucleoside (34 mmol) was evaporated twice from 100ml anhydrous pyridine and dried *in vacuo*. The resultant residue was dissolved in 150 ml anhydrous pyridine, cooled to 0°C in an ice water bath, and 37 mmol added. After stirring for 15 min. at 0°C the ice bath was removed and the reaction stirred for 4-5 hours at room temperature. The mixture was concentrated *in vacuo* to an oil and taken up in 100 ml of methylene chloride. The solution was washed successively with saturated sodium bicarbonate (25 ml), water (25 ml), and saturated sodium chloride (25 ml). The organic phase was dried over anhydrous sodium sulfate and the solvent removed by rotary evaporation. The reaction product was purified by silica gel chromatography, eluting with 10% (v/v) methanol in methylene chloride. Fractions containing the major product were pooled and dried *in vacuo*.

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-2'-deoxythymidine: yield 60%; R_f= 0.47 (silica, methylene

chloride/methanol 9:1); ^{1}H NMR ($CDCl_3$): 1.7 (d, 3H) CH_3; 1.9 (s, 3H) CH_3; 2.0-2.4 (m, 2H) 2'-CH_2; 4.1-4.5 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 6.1 (m, 2H) O-CH_2-O; 6.3 (m, 2H) 1'-H, CH (benzyl); 7.0 and 7.5 (s and m, 2H) aromatic; 7.35 (s, 1H) 6H. MS

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-N6-phenoxyacetyl-2'-deoxyadenosine: yield 52%; R_f= 0.69 (silica, methylene chloride/methanol 9:1); ^{1}H NMR ($CDCl_3$): 1.65 (d, 3H) CH_3; 2.6 and 2.9 (m, 2H) 2'-CH_2; 4.2-4.4 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 4.9 (d, 2H) CO-CH_2-O; 6.25 (m, 1H) 1'-H; 6.5 (q, 1H) CH (benzyl); 7.05 (m, 4H) aromatic (PAC), aromatic (nitrobenzyl); 7.35 (m. 2H) aromatic (PAC); 7.45 (s, 1H) aromatic (nitrobenzyl); 8.25 and 8.75 (d, 2H) 2H, 8H; 9.4 (bs, 1H) NH.

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-N2-phenoxyacetyl-2'-deoxyguanosine: yield 70%; R_f= 0.57 (silica, methylene chloride/methanol 6:1); 1H NMR ($CDCl_3$/CD_3OD): 1.6 (dd, 3H) CH_3; 2.4-2.8 (m, 2H) 2'-CH_2; 4.2-4.6 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 4.7 (s, 2H) CO-CH_2-O; 6.1-6.4 (m, 4H) O-CH_2-O, 1'-H, CH (nitrobenzyl); 6.9-7.15 (m, 4H) aromatic (PAC), aromatic (nitriobenzyl); 7.3-7.42 (m, 3H) aromatic (PAC), aromatic (nitrobenzyl); 8.1 (s, 1H) 8H. MS

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-N4-isobutyryl-2'-deoxycytidine: yield 60%; R_f= 0.56 (silica, methylene chloride/methanol 9:1); 1H NMR ($CDCl_3$): 1.2 (dd, 6H) 2CH_3 (ibu); 1.65 (dd, 3H) CH_3, 2.1 (m, 1H) 2'-H; 2.5-2.8 (m, 2H) 2'-H, CH (ibu); 4.2-4.5 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 6.1 (m, 2H) O-CH_2-O; 6.3 (m, 2H) 1'-H, CH (benzyl); 7.0 and 7.49 (d, 2H) aromatic; 7.45 and 8.0 (dd, 2H) 5H, 6H; 8.35 (bs, 1H) NH. MS

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-2'-deoxynucleoside 3'-O-(2-cyanoethyl)-N,N'-diisopropylaminophosphites were prepared as follows. 5'-protected deoxynucleoside (10 mmol) was dissolved in 75 ml of methylene chloride. Diisopropylethylamine (30 mmol) was added and the solution cooled to 0°C in an ice water bath. 2-cyanoethyl-N,N'-diisopropylchlorophosphoramidite (25 mmol) was slowly added to the cooled mixture. The ice bath was removed

and the reaction stirred for 2 hours at room temperature. Anhydrous methanol (5 ml) was added and the reaction stirred for an additional 30 min. at room temperature. The mixture was diluted with methylene chloride (150 ml) and washed with saturated sodium bicarbonate (50 ml) followed by water (50 ml). The organic phase was dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The product was purufied by silica gel chromatography, eluting with 45:45:10 ethyl acetate/methylene chloride/triethylamine. Fractions containing the major product were pooled and dried *in vacuo*.

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-2'-deoxythymidine 3'-O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite: yield 80%; R_f= 0.58 (silica, 45:45:10 ethyl acetate/methylene chloride/triethylamine); ^1H NMR ($CDCl_3$): 1.1-1.3 (m, 12H) CH_3 (isopropyl); 1.65 (d, 3H) CH_3 (nitrobenzyl); 1.9 (s, 3H) CH_3; 2.1-2.3 (m, 1H) 2'-H; 2.4-2.7 (m, 3H) 2'-H, CH_2-CN; 3.5-3.9 (m, 4H) NH (isopropyl), P-O-P-CH_2; 4.1-4.5 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 6.15 (s, 2H) O-CH_2-O; 6.3 (m, 2H) 1'-H, CH (nitrobenzyl); 7.0 and 7.5 (s, 2H) aromatic; 7.3 (m, 1H) 6H; 8.4 (bs, 1H) NH (thymidine). MS

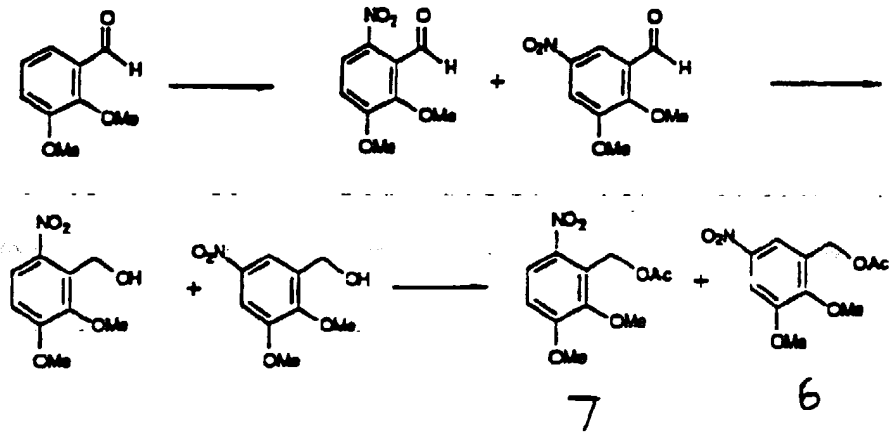
5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-N4-isobutyryl-2'-deoxycytidine 3'-O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite: yield 85%; R_f= 0.52 (silica, 45:45:10 ethyl acetate/methylene chloride/triethylamine); ^1H NMR ($CDCl_3$): 1.1-1.3 (m, 18H) CH_3 (isopropyl), CH_3 (ibu); 1.65 (m, 3H) CH_3 (nitrobenzyl); 2.1 (m, 1H) 2'-H; 2.55-2.8 (m, 3H) 2'-H, CH_2-CN; 3.5-3.9 (m, 4H) NH (isopropyl), P-O-CH_2; 4.2-4.5 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 6.1-6.35 (m, 4H) O-CH_2-O, 1'-H, CH (nitrobenzyl); 7.0 and 7.5 (d, 2H) aromatic; 7.4 and 7.95 (m, 2H) 5-H, 6H; 8.25 (bs, 1H) NH (amide). MS

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-N6-phenoxyacetyl-2'-deoxyadenosine 3'-O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite: yield 81%; R_f= 0.50 (silica, 45:45:10 ethyl acetate/methylene chloride/triethylamine); ^1H NMR ($CDCl_3$): 1.1-1.3 (m, 12H) CH_3 (isopropyl); 1.65 (m, 3H) CH_3 (nitrobenzyl); 2.6-3.0 (m, 4H) 2'-H, CH_2-CN; 3.6-3.9 (m, 4H) NH (isopropyl), P-O-CH_2; 4.3-4.6 (m, 4H) 3'-H, 4'-H, 5'-

CH_2; 4.7 (2s, 2H) $CO-CH_2-O$; 6.1 (s, 2H) $O-CH_2-O$; 6.3 (m, 1H) 1'-H; 6.5 (m, 1H) CH (nitrobenzyl); 7.1 (m, 4H) aromaitc (nitrobenzyl), aromatic (PAC); 7.35 (m, 2H) aromatic (PAC); 7.45 (s, 1H) aromatic (nitrobenzyl); 8.2 and 8.8 (s, 2H) 2H, 8H; 9.4 (bs, 1H) NH (amide). MS

5'-O-(α-methyl-6-nitropiperonyloxycarbonyl)-N2-phenoxyacetyl-2'-deoxyguanosine 3'-O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite: yield 75%; R_f= 0.55 (silica, 9:1 methylene chloride/methanol); ^1H NMR ($CDCl_3$): 1.3 (d, 12H) CH_3 (isopropyl); 1.6 (dd, 3H) CH_3 (nitrobenzyl); 2.5-2.9 (m, 4H) 2'-H, CH_2-CN; 3.55-3.95 (m, 4H) NH (isopropyl), $P-O-CH_2$; 4.3-4.6 (m, 4H) 3'-H, 4'-H, 5'-CH_2; 4.7 (2s, 2H) $CO-CH_2-O$; 6.1-6.35 (m, 4H) $O-CH_2-O$, 1'-H, CH (nitrobenzyl); 6.95-7.15 (m, 4H) aromaitc (nitrobenzyl), aromatic (PAC); 7.35-7.45 (m, 3H) aromatic (PAC), aromatic (nitrobenzyl); 7.9 (s, 1H) 8H. MS

Example 6

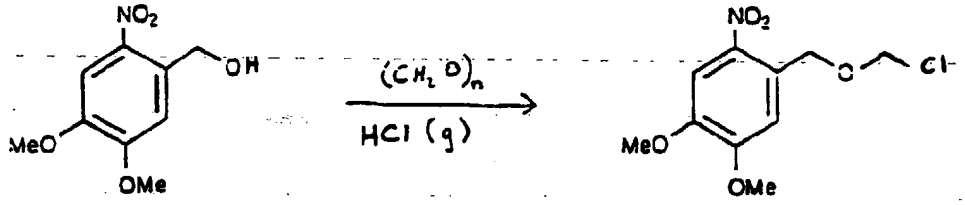


2,3-Dimethoxybezaldehyde (1.00 g; 6.02 mmol) was added to 10 mL of 70% HNO_3 cooled to 0°C. The solution was warmed to room temperature after 5 min and stirring was continued for an additional 10 min. The reaction mixture was poured into cold water and filtered to give 1.16 g of an off-white solid precipitate. The crude 1:1 mixture of regioisom rs was used without further purification.

The crude nitrobenzaldehyde was reduced by treating a solution of the aldehyde (501 mg; 2.37 mmol) in 25 mL of anhydrous THF with $NaBH_4$ (185 mg; 4.89 mmol) cooled to 15°C. After stirring the solution for 10 min., 3 mL of MeOH was added to aid solubility. The reaction mixture was stirred an additional 30 min. and was then partitioned between EtOAc and sat. NH_4Cl. The organic phase was washed (sat. NaCl), dried ($MgSO_4$), and evaporated to give 0.56 g of a 1:1 mixture of the two regioisomers as a white crystalline solid. The alcohols were used without further purification.

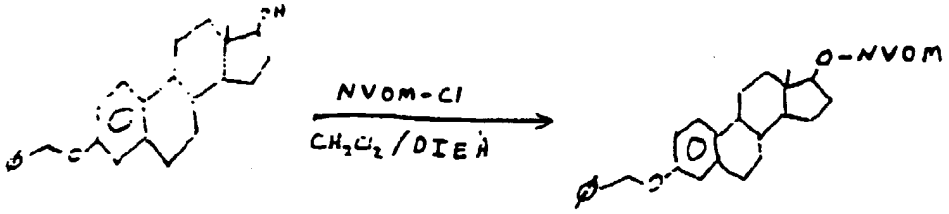
A solution of the crude alcohols (0.56 g) in 10 mL of $CHCl_3$ was treated with 5 mL of pyridine and 2 mL of Ac20 for 3 hours at room temperature. The reaction mixture was partitioned between CHCl3 and 1 NHCl, and the organic phase was dried ($MgSO_4$) and evaporated to give .6 g of a colorless oil. Chromatography on silica gel with 25% EtOAc in hexanes afforded 314 mg of 6 as a white solid (52% yield for the two steps) and 261 mg of 7 as a white solid (43% yield for the two steps).

Example 7



Nitroveratryloxymethylchloride (NVOM-Cl) was prepared as follows. To a suspension/solution of 5g (0.0235 mol) of NV-OH in 50 ml of toluene was added 1.0g (0.033 mol) dry powdered paraformaldehyde [$(CH_2O)_n$] with vigorous stirring. HCl(g) was bubbled into the solution (no cooling) until all of the solid had gone into solution (30 min) at which time the solution turned dark green with a black aqueous layer coating the walls of the flask. The mixture was then allowed to stand at room temperature for approximately 30 min and the green solution was then decanted off the black aqueous

layer. To the toluene solution was added 30 ml of toluene and 20 ml of hexane followed by 2.5 g of $MgSO_4$ (anhydrous). The solution was stirred at approximately 10°C for 15 min, filtered, and the filtrate rotary evaporated (at approximately 30°C) to an amber oil. The oil was placed on a vacuum line whereupon yellow crystals began to come out of the oil. The semi-solid was then triturated 3 times with 40 ml of hexane each time decanting off the hexane. The yellow crystalline residue was then dried in vacuum at room temperature. The yield was 5.67 g on 92%.

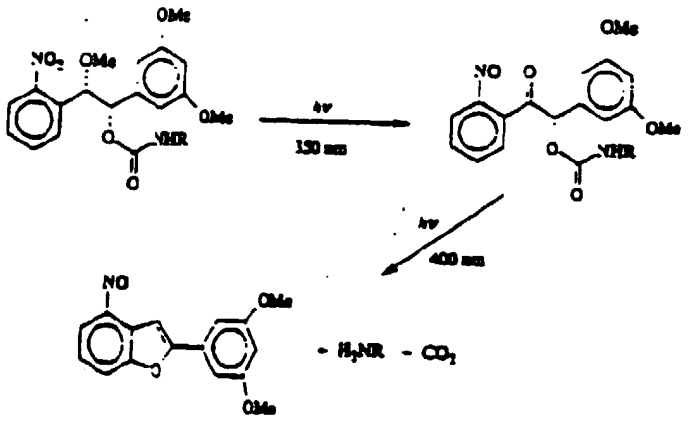


An NVOM β-estradiol-benzylether was prepared as follows. To a mixture of 400 mg (1.105 mmol) of β-estradiol-3-benzyl ether and 434 mg (1.66 mmol) of NVOM-Cl in 5 ml of CH_2Cl_2 was added 213 mg (1.65 mmol) of DIE. After stirring overnight at room temperature the mix was with H_2O, 0.1N HCl, dried (Na_2SO_4) and solvent removed. The yield was 584 mg or 90%. NVOM protected 2-deoxyadenosine was prepared by an analogous method.

VI. Multiple-quantum Protecting Groups

In most of the above described compounds, only a single photon is required (in theory) to photolyze a single molecule of the compound. However, some photoreactive groups of this invention require two or more photons for photolysis. Such compounds may be useful during light-directed synthesis on substrates to prevent unwanted deprotections caused by the diffraction or light around the edges of the mask (see the discussion of binary synthesis strategies above). Compounds useful for this type of "multiple quantum" deprotection include those in which a first photon alters the photoactive

isomerization, redox reaction, cyclization, or deprotection of a functional group on the protecting group itself. Upon exposure to the second photon, the altered protecting group is then removed. One example of such a deprotection is shown below:



It will be appreciated by those skilled in the art that the compound shown in the example above is but one of many compounds which require two photons to become deprotected.

VII. Photolysis of Protected Group Compounds

Removal of the protecting group is accomplished by irradiation to separate the reactive group and the degradation products derived from the protecting group. Not wishing to be bound by theory, it is believed that irradiation of NVOC- and MeNVOC-protected oligomers, for example, occurs by the following reaction schemes:

NVOC-AA -> 3,4-dimethoxy-6-nitrosobenzaldehyde + CO_2 + AA
MeNVOC-AA-> 3,4-dimethoxy-6-nitrosoacetophenone + CO_2 +AA

where AA represents the N-terminus of an amino acid or oligomer.

Along with the unprotected amino acid, other

3,4-dimethoxy-6-nitrosophenylcarbonyl compound, which can react with nucleophilic portions of the oligomer to form unwanted secondary reactions. In the case of an NVOC-protected amino acid, the degradation product is a nitrosobenzaldehyde, while the degradation product for MeNVOC is a nitrosophenyl ketone. It is believed that the product aldehyde from NVOC degradation reacts with free amines to form a Schiff base (imine) that affects the remaining polymer synthesis. Preferred photoremovable protecting groups react slowly or reversibly with the oligomer on the support.

Not wishing to be bound by theory, it is believed that the product ketone from irradiation of a MeNVOC-protected oligomer reacts at a slower rate with nucleophiles on the oligomer than the product aldehydes from irradiation of the same NVOC-protected oligomer. Although not unambiguously determined, it is believed that this difference in reaction rate arises from radical stabilization. However, it may also be due to the difference in general reactivity between aldehydes and ketones towards nucleophiles due to steric and electronic effects.

In some preferred embodiments, scavengers or other reagents are added to the reaction mixture to react with and render harmless photolysis byproducts that might otherwise react with a growing oligomer. Suitable scavengers (which are often nucleophiles) will be known to those of skill in the art. Specific examples include acids, bisulfites, hydroxy-containing compounds, amines, etc.

Because photolysis is a first order process, the protected compound must be illuminated for nine half-lives to effect 100% removal. Very fast deprotection rates are therefore desired because they lessen the overall exposure time, reducing the effects of stray light and the total time necessary to synthesize a polymer.

The photolysis of N-protected L-phenylalanine in solution having different photoremovable protecting groups was analyzed, and the results are presented in the following table:

Photolysis of Protected L-Phe-OH

	$t_{1/2}$ in seconds			
Solvent	NBOC	NVOC	MeNVOC	MeNPOC
Dioxane	1288	110	24	19
5mM H_2SO_4/Dioxane	1575	98	33	22

The half life, $t_{1/2}$ is the time in seconds required to remove 50% of the starting amount of protecting group. The photolysis was carried out in the indicated solvent with 362/364 nm-wavelength irradiation having an intensity of 10 mW/cm^2, and the concentration of each protected phenylalanine was 0.10 mM.

The table shows that deprotection of NVOC-, MeNVOC-, and MeNPOC-protected phenylalanine proceeded faster than the deprotection of NBOC. Furthermore, it shows that the deprotection of the two derivatives that are substituted on the benzylic carbon, MeNVOC and MeNPOC, were photolyzed at the highest rates in both dioxane and acidified dioxane.

Another photolysis study was performed on various MeNPOC nucleosides. The $t_{1/2}$ rates for the disappearance of starting material are tabulated below and are normalized to a photolysis intensity of 10 mW/cm^2 power using the 350-450 nm dichroic reflector on a Hg(Xe) arc lamp. DTT was intended to act as scavenger for the nitrosoketone byproduct and was included in the study to see if it had any effect on the photolysis rates.

Table II

($t_{1/2}$ in seconds)

Nucleoside	Dioxane	Acid/Diox	CH_3CN	DTT/CH_3CN
NVOC-Ac	93.4			
MeNPOC-A(PheAc)	32.1	decomp.	73.1	74.7
MeNPOC-C(i-Bu)	31.3	39.0	80.6	90.5
MeNPOC-G(PheAc)	37.9	decomp.	80.7	79.4
MeNPOC-G(iPrPheAc)	41.0	decomp.	93.1	88.0

In another photolysis study, a 0.1mM solution of each of the four 5'-protected nucleosides prepared in Example 5, MeNPoc-dT, MeNPoc-dC , MeNPoc-dG , and MeNPoc-dA was prepared in dioxane. A 200 μL aliquot of one of the four deoxynucleoside solutions was irradiated with 350-450nm light at 14.5 mW/cm^2 (Oriel) in a narrow path (2mm) quartz cuvette for a given period of time x_1. A fresh aliquot of the 0.1mM solution was irradiated as before for a different time x_2. Four or five time points were collected for each MeNPoc-deoxynucleosides.

Each time point was analyzed for loss of starting material at 280 nm on a nucleosil 5-C_8 HPLC column using a mobile phase of 60% (v/v) CH_3CN in water containing 0.1% (v/v) TFA. MeNPOC-dT required a mobile phase of 70% (v/v) methanol in water. The integrated peak area of the remaining 5'-MeNPoc-2'-deoxynucleoside was calculated. A plot of natural logarithm of area vs. time resulted in a straight line of slope k where $t_{1/2} = 0.639/k$.

Table III. Photolytic half-lives of 5'-O-MenPoc-2'-deoxynucleosides

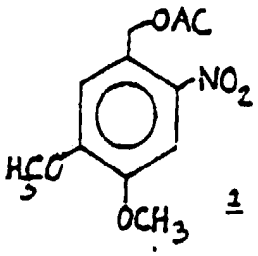
Compound	$t_{1/2}$ (sec.)
5'-O-MenPoc-2'-deoxythymidine	28
5'-O-MenPoc-N4-isobutyryl-2'-deoxycytidine	31
5'-O-MenPoc-N6-phenoxyacetyl-2'-deoxyadenosine	18
5'-O-MenPoc-N2-phenoxyacetyl-2'-deoxyguanosine	27

In other photolysis experiments, the deprotection of nitroveratryloxymethyl protected compounds was shown to compare favorably with certain NVOC and MeNPOC compounds.

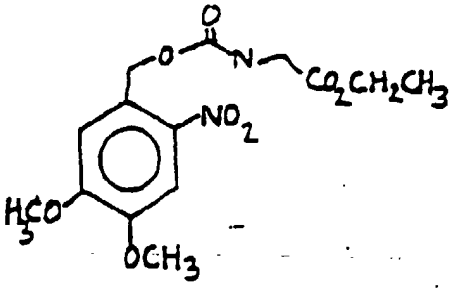
Ta le IV

M DS DIOX NE 5mM 5mM H_3 I:

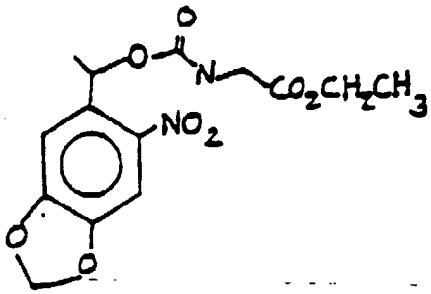
	λ nm	t1/2 (s)	t1/2 (s)	t1/2 (s)	t1/2 (s)	ε	mM⁻¹cm⁻¹
1	365	131	129	102	315	4.21410
2	365	79.2	97.1	69.7	247	4.77410
3	365	35.3	39.7	29.6	78.8	3.14310
4	365	10.4	13.5	11.1	30.0	4.94410
5	365	13.1	13.0	10.0	36.5	5.09710



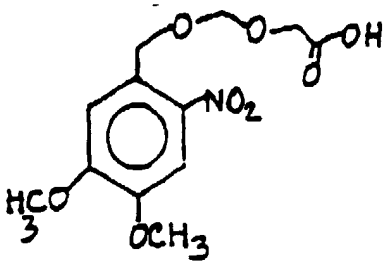
1



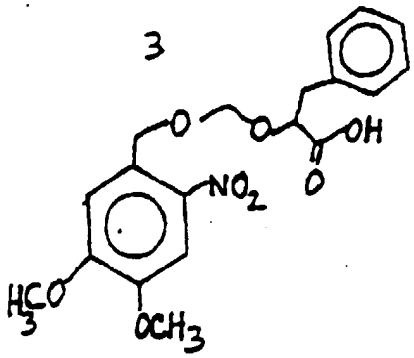
2



3



4



5

Still other photolysis results are shown below. In the second solvent, 5mM H_2SO_4 is used and in the third solvent 5mM DTT is used. The photolysis radiation was provided at a wavelength of 365 nm and an intensity of 10 mW/cm^2. The

listed values in the chart are t$_{\frac{1}{2}}$ in seconds. The various compounds are shown in Fig. 6.

Table V

Compd.	Dioxane	H_2SO_4/ Dioxane	DTT/ Dioxane	CH_3CN	Extinction Coeff.
1	1785	1941	1446	717	65
2	2088	807	828	507	801
3	132	129	102	315	4214
4	135	65	107	285	3810
5	582	242	261	528	2411
6	216	213	217	201	1248
7	441	72	289	849	3401
8	3540	2667	1602	2487	1273
9	265	280	241	184	305
10	36	42	30	83	4092
11	35	33	29	65	3103
12	60	58	73	50	1321
13	103	95	46	48	2066
14	393	207	47	244	7140
15	1644	1740	2802	3720	871

VIII. Basis Sets of Photoprotected Amino or Nucleic Acids

The deprotection rate (or photolysis half life) of a protected compound is a function of the particular photosensitive protecting group as well as the amino or nucleic acid functional group being protected. It is also a function of the solvent in which photolysis is performed and

neutralize photolysis decomposition products. As illustrated in the above Tables, the deprotection rate can vary substantially for the basis set of naturally occurring deoxynucleosides, each protected with the same photosensitive group. It has also been observed that the set of NVOC-protected amino acids (naturally occurring) have photolysis half lives ranging from 70-150 seconds.

In VLSIPS™, it is desirable that the protected compounds are deprotected at substantially the same rate to prevent unwanted side reactions between the byproducts described above and the unprotected amino acids. In other words, monomers that deprotect earliest are exposed to reactive photolysis byproducts until the substrate is washed. If all oligomers on the substrate deprotect at the same rate, the time of exposure will be minimized. In VLSIPS™, it is also desirable to minimize the exposure time so that stray light from an illuminated region does not deprotect compounds in adjacent regions. To ensure a minimal exposure time, it is preferred to match the various photoreactive protecting groups of the invention with different amino acids (or other monomer types) so that all of the amino acids will be deprotected at substantially the same rate in a particular solvent. Sets of such rate-matched photoprotected amino acids or nucleosides are hereinbelow referred to as "basis sets". It will be appreciated that basis sets can be created for any class or subclass of compounds, most notably the natural or unnatural amino acids and the natural or unnatural nucleic acids.

"At substantially the same rate" is defined herein to mean that the half-life for deprotection of the most rapidly deprotected compound in the basis set is within about 25% of the compound with the slowest deprotection half-life in a particular solvent. Preferably, the differences between half-lives is about 10% and most preferably about 5%.

One preferred class of compounds for forming a basis set is a plurality of the twenty naturally-occurring amino acids. Another preferred class is a subset of the D-isomers of the twenty naturally-occurring amino acids. Yet another

include β amino acids and amino acids whose side chains have been altered in order to adjust the stearic bulk, hydrophobicity, or electronic character of the corresponding natural amino acid. Such amino acid analogs have found increasing utility in drug research (see, e.g., Fauchère).

Still another preferred class is formed by the common nucleosides adenosine, thymidine, guanosine, cytidine, and inosine in addition to their 2', or 3'-deoxy analogs and their 2', 3', or 5'-phosphates. "Phosphate" is meant herein to include the phosphotriesters (-OP(OR")(OR‴)), phosphoramidites (-OP(OR")(NRR'), where R-R‴ are described above), as well as phosphate anions ($-OP(O)O_2H^-$, and $-OP(O)O_2^{-2}$).

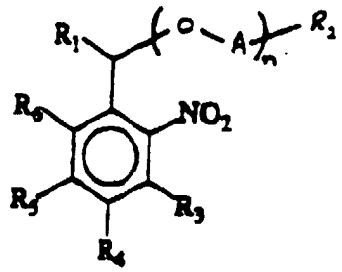
Typically, the choice of protecting groups will depend on the character of the solvent which, as described above, has an important effect on the rate of deprotection. One preferred solvent for both amino acids and nucleosides is dioxane. Another preferred solvent is acetonitrile.

IX. Conclusion

The above description is illustrative and not restrictive. Many variations of the invention will become apparent to those of skill in the art upon review of this disclosure. Merely by way of example, while the invention is illustrated primarily with regard to peptide and nucleotide synthesis, the invention is not so limited. The scope of the invention should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the appended claims along with their full scope of equivalents.

WHAT IS CLAIMED IS:

1. A compound having the formula:



wherein n is 0 or 1; A is -C(O)-, -(CQ_1Q_2)-, or -C(S)-; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_2 is a functional group of a molecule selected from the group consisting of natural or unnatural amino acids and peptides, nucleosides, nucleoside analogs, and oligonucleotides; R_3-R_6 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl, and cyclic bridges between adjacent substituents where the bridges are selected from the group consisting of acetals, ketals, orthoesters, thioesters, fused aromatic groups, and ether groups; provided that when n is 0 or A is -C(O)- (i) R_4 and R_5 are not both methoxy when R_1, R_3, and R_6 are hydrogen; (ii) R_1 is not hydrogen, methyl, phenyl, or 2-nitrophenyl when R_3-R_6 are hydrogen simultaneously; and (iii) R_1 is not 2-nitrophenyl or 3,4-dimethoxy-6-nitrophenyl when R_3 and R_6 are hydrogen and R_4 and R_5 are both either hydrogen or methoxy.

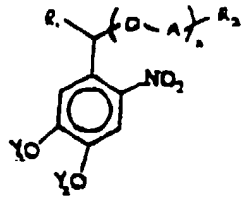
2. The compound of claim 1, wherein said functional group is a carboxyl, hydroxyl, thiol or amino group.

3. The compound of claim 1, wherein R_2 has the formula -NRR' where R is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, acyl, or benzyl, and R' is the residue of a natural or unnatural amino acid.

4. The compound of claim 3, wherein R is methyl.

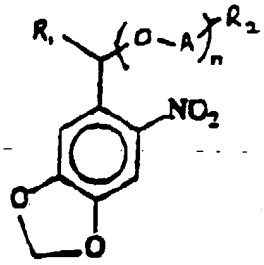
5. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 and R_6 are hydrogen, and R_4 and R_5 are methoxy.

6. The compound of claim 1 having the following structure:

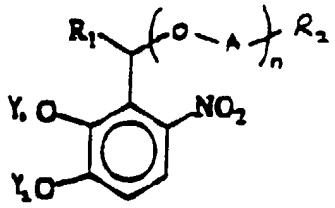


wherein R_1 is hydrogen or methyl, R_3 and R_6 are hydrogen, and Y_1 and Y_2 are independently C_1-C_8 alkyl groups, or together form a cyclic bridge acetal or ketal.

7. The compound of claim 6, wherein R_4 and R_5 form a methylenedioxy acetal ring.

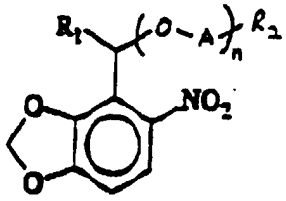


8. The compound of claim 1 having the following structure:

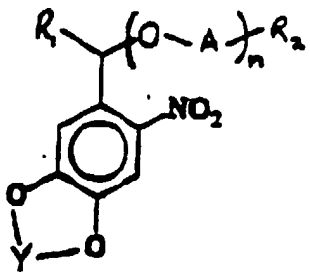


wherein R_1 is hydrogen or methyl, R_3 and R_6 are hydrogen, and Y_1 and Y_2 are independently C_1-C_8 alkyl or together form a cyclic bridge acetal or ketal.

9. The compound of claim 8, wherein R_5 and R_6 form a methylenedioxy acetal ring.

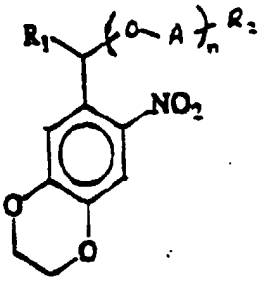


10. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 and R_6 are hydrogen, and R_4 and R_5 together form a ring, the compound having the structure



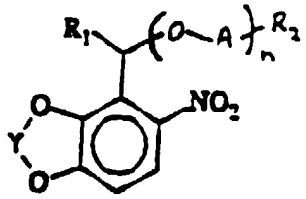
wherein Y is -CRR'- or -CRR'-CR"R"'-, and where R, R', R" and R"' are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, benzyl, alkoxy, aryloxy, carboxy, alkyl ester, R and R' together are the oxygen of a carbonyl group, and R" and R"' together are the oxygen of a carbonyl group.

11. The compound of claim 10, wherein Y is -CRR'-CR"R"'- and R, R', R" and R"' are hydrogen.



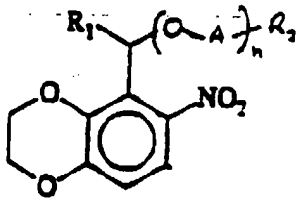
12. The compound of claim 10, wherein Y is -CRR'- and R is hydrogen and R' is alkoxy, aryloxy, carboxy, or alkyl ester.

13. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 and R_4 are hydrogen, and R_5 and R_6 together form a ring, the compound having the structure



wherein Y is -CRR'- or -CRR'-CR"R"'-, and where R, R', R" and R"' are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, benzyl, alkoxy, aryloxy, carboxy, alkyl ester, R and R' together are the oxygen of a carbonyl group, and R" and R"' together are the oxygen of a carbonyl group.

14. The compound of claim 13, wherein Y is -CRR'-CR"R"'- and R, R', R" and R"' are hydrogen.



15. The compound of claim 13, wherein Y is -CRR'- and R is hydrogen and R' is alkoxy, aryloxy, carboxy, or alkyl ester.

16. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 and R_4 are hydrogen, and R_5 and R_6 are methoxy.

17. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 is dimethylamino, R_4 is hydrogen, and R_5 and R_6 are methoxy.

18. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 and R_5 are hydrogen, and R_4 is dimethylamino, and R_5 is methoxy.

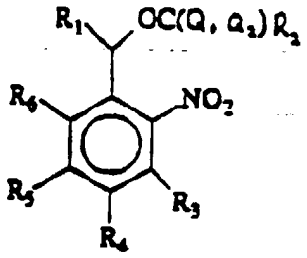
19. The compound of claim 1, wherein R_1 is hydrogen or methyl, R_3 and R_5 are hydrogen, and R_4 is methoxy, and R_5 is dimethylamino.

20. The compound of claim 1 wherein R_2 has the formula -NRR' where R is either hydrogen, methyl, or acetyl, and R' is a natural or unnatural amino acid residue.

21. The compound of claim 1 wherein n is 1 and A is $-CH_2-$.

22. The compound of claim 21, wherein said functional group is carboxyl, amino, thiol, amide or hydroxyl.

23. A compound having the formula:



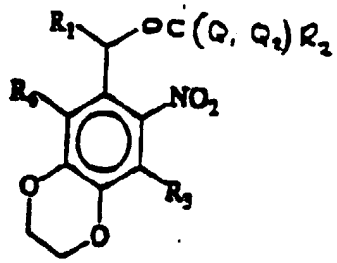
wherein R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_6 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_2 is a molecule functional group selected from the group consisting of amines, carboxyl groups, thiols, imidazoles, amides, and hydroxy groups; R_3-R_6 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_2 alkyl, aryl, or benzyl, and cyclic bridges between adjacent substituents where the bridges are selected from the group consisting of acetals,

ketals, orthoesters, thioesters, fused aromatic groups, and ether groups.

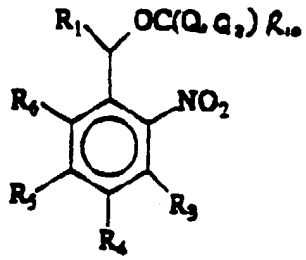
24. The compound of claim 23 wherein R_1, Q_1, and Q_2 are hydrogen.

25. The compound of claim 23 wherein the functional group is part of a molecule selected from the group consisting of nucleosides, nucleoside analogs, oligonucleotides, amino acids, and peptides.

26. The compound of claim 23 having the following structure:



27. A compound having the formula:



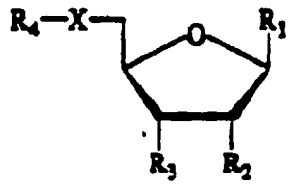
wherein R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_{10} is a group selected from the group consisting of halo, hydroxyl, tosyl, mesyl, trifluoromethyl, diazo, azido; R_3-R_5 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl, and cyclic bridges between adjacent substituents where the bridges are selected

from the group consisting of acetals, ketals, orthoesters, thioesters, fused aromatic groups, and ether groups.

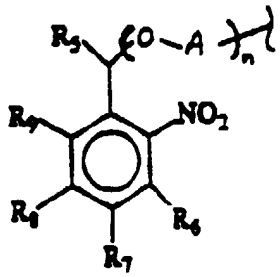
28. The compound of claim 27 wherein R_{10} is a halide.

29. The compound of claim 28 wherein R_1, Q_1, and Q_2 are all hydrogen; R_{10} is chloro; R_3 and R_6 are both hydrogen; and R_4 and R_5 are both methoxy.

30. A compound having the formula:



wherein X is selected from the group consisting of oxygen or sulphur; R_1 is a purine, a pyrimidine, or an analog thereof; and R_2, R_3, and R_4 are each independently hydrogen, hydroxy, oligonucleotide, alkoxy, tetrahydropyranyl, β-benzoylpropionyl, acetyl, a bridge between adjacent substituents which together form an acetal, ketal, orthoester, or cyclic ether, a photolabile group, -NRR', $-OP(O)O_2^{-2}$, $-OP(O)O_2H^-$, -OP(O)(OR")(OR"'), -OP(OR")(NR"R"') where R and R' are independently selected from the group consisting of C_1-C_3 alkyl, aryl, benzyl, or acetyl, and R" and R"' are selected independently from the group consisting of C_1-C_3 alkyl, aryl, benzyl, or C_1-C_3 cyanoalkyl; provided that at least one of R_2-R_4 is a photoreactive compound and wherein said photolabile group having the formula:



wherein n is 0 or 1; A is -C(O)-, $-(CQ_1Q_2)-$, or -

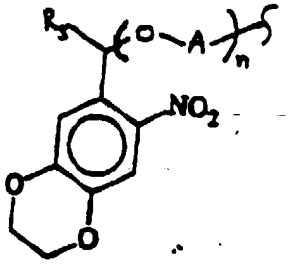
aryl, alkoxy, aryloxy, or carboxy; R_5-R_9 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl, and cyclic bridges between adjacent substituents where the bridges are selected from the group consisting of acetals, ketals, orthoesters, thioesters, fused aromatic groups, and ether groups.

31. The compound of claim 30, wherein R_4 is said photolabile group.

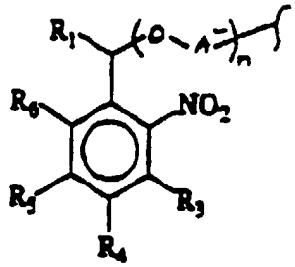
32. The compound of claim 30, wherein R_2 is said photolabile group.

33. The compound of claim 30, wherein R_3 is said photolabile group.

34. The compound of claim 30, wherein X is oxygen and said photolabile group has the formula:



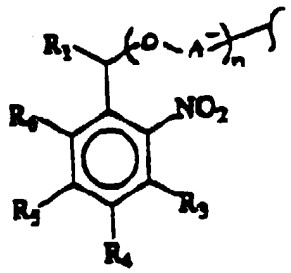
35. A basis set of protected amino acids, comprising a plurality of natural or unnatural amino acids, each independently coupled through either an amine or carboxyl group to at least one photolabile group having the formula:



wherein n is 0 or 1; A is -C(O)-, -(CQ_1Q_2)-, or -C(S)-; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_3-R_5 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl, and cyclic bridges between adjacent substituents where the bridges are selected from the group consisting of acetals, ketals, orthoesters, thioesters, fused aromatic groups, and ether groups; wherein said photolabile groups are chosen so that each member of the basis set photolyzes at substantially the same rate upon exposure to radiation in a particular solvent.

36. The basis set of claim 35, wherein said particular solvent is dioxane.

37. A basis set of protected nucleosides or nucleoside analogs comprising a plurality of nucleosides or nucleoside analogs, each independently coupled to a photolabile group having the formula:



wherein n is 0 or 1; A is -C(O)-, -(CQ_1Q_2)-, or -C(S)-; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_3-R_5 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl, and cyclic bridges between adjacent substituents where the bridges are selected from the group consisting of acetals, ketals, orthoesters,

said photolabile groups are chosen so that each member of the basis set photolyzes at substantially the same rate upon exposure to radiation in a particular solvent.

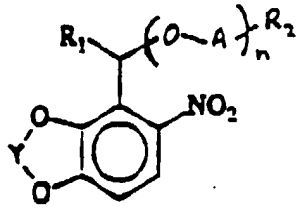
38. The basis set of claim 37, wherein said particular solvent is acetonitrile.

39. The basis set of claim 37, wherein said nucleosides are selected from the group consisting of 2'-deoxynucleosides, 3'-deoxynucleosides and 2',3'-dideoxynucleosides.

40. The basis set of claim 37, wherein said nucleosides include a phosphate-containing group selected from the group consisting of $-OP(O)O_2^{-2}$, $-OP(O)O_2H^-$, $-OP(OR'')(OR''')$, and $-OP(OR'')(NR''R'')$, wherein R and R' are independently C_1-C_3 alkyl, aryl, benzyl, or acetyl, and R'' and R''' are selected independently from the group consisting of C_1-C_3 alkyl, aryl, benzyl; or C_1-C_3 cyanoalkyl.

41. The basis set of claim 40, wherein said phosphorous containing group is located at a position selected from the group consisting of the 2'-carbon, the 3'-carbon and the 5'-carbon positions of said nucleoside or nucleoside analog.

42. A compound having the formula:



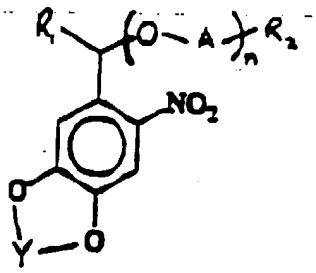
wherein n is 0 or 1; A is $-C(O)-$, $-(CQ_1Q_2)-$, or $-C(S)-$; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_9 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_2 is a molecule functional group selected from the group consisting of amines, carboxyl

R_3 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl; and wherein Y is -CRR'- or -CRR'-CR"R"'-, and where R, R', R" and R"' are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, benzyl, alkoxy, aryloxy, carboxy, alkyl ester, R and R' together are the oxygen of a carbonyl group, and R" and R"' together are the oxygen of a carbonyl group.

43. The compound of claim 42 wherein R_1, Q_1, and Q_2 are hydrogen.

44. The compound of claim 42 wherein the functional group is part of a molecule selected from the group consisting of nucleosides, nucleoside analogs, oligonucleotides, amino acids, and peptides.

45. A compound having the formula:



wherein n is 0 or 1; A is -C(O)-, -(CQ_1Q_2)-, or -C(S)-; R_1, Q_1, and Q_2 are independently hydrogen, C_1-C_8 alkyl, aryl, alkoxy, aryloxy, or carboxy; R_2 is a molecule functional group selected from the group consisting of amines, carboxyl groups, thiols, imidazoles, amides, and hydroxy groups; R_3 and R_5 are independently hydrogen, alkoxy, aryloxy, benzyloxy, acyloxy, nitro, alkylthio, arylthio, hydroxyl, halogen, -NR'R" where R' and R" are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, or benzyl; and

R‴ are selected independently from the group consisting of hydrogen, C_1-C_8 alkyl, aryl, benzyl, alkoxy, aryloxy, carboxy, alkyl ester, R and R' together are the oxygen of a carbonyl group, and R" and R‴ together are the oxygen of a carbonyl group.

46. The compound of claim 45 wherein R_1, Q_1, and Q_2 are hydrogen.

47. The compound of claim 45 wherein the functional group is part of a molecule selected from the group consisting of nucleosides, nucleoside analogs, oligonucleotides, amino acids, and peptides.

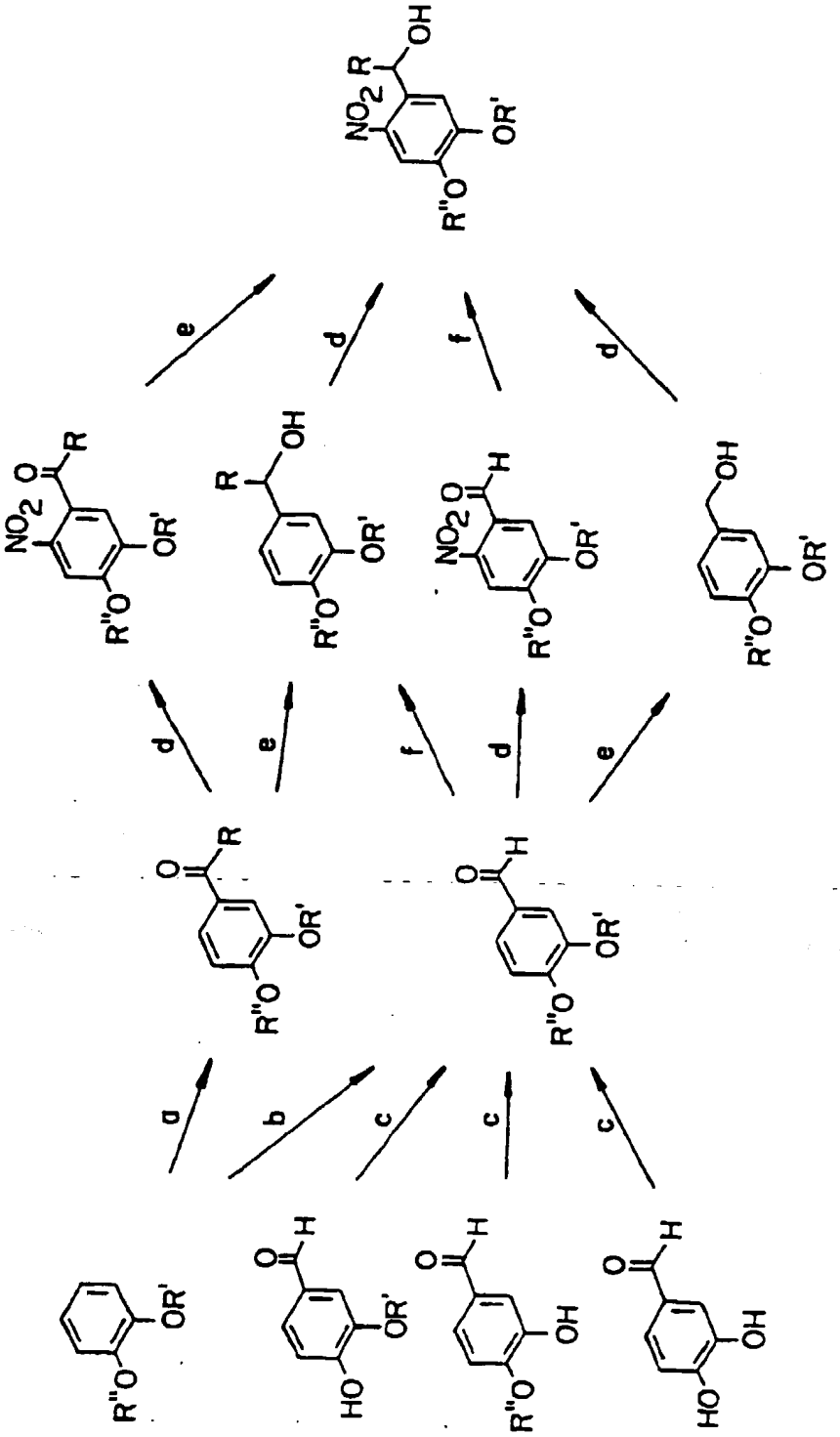


FIG. 1

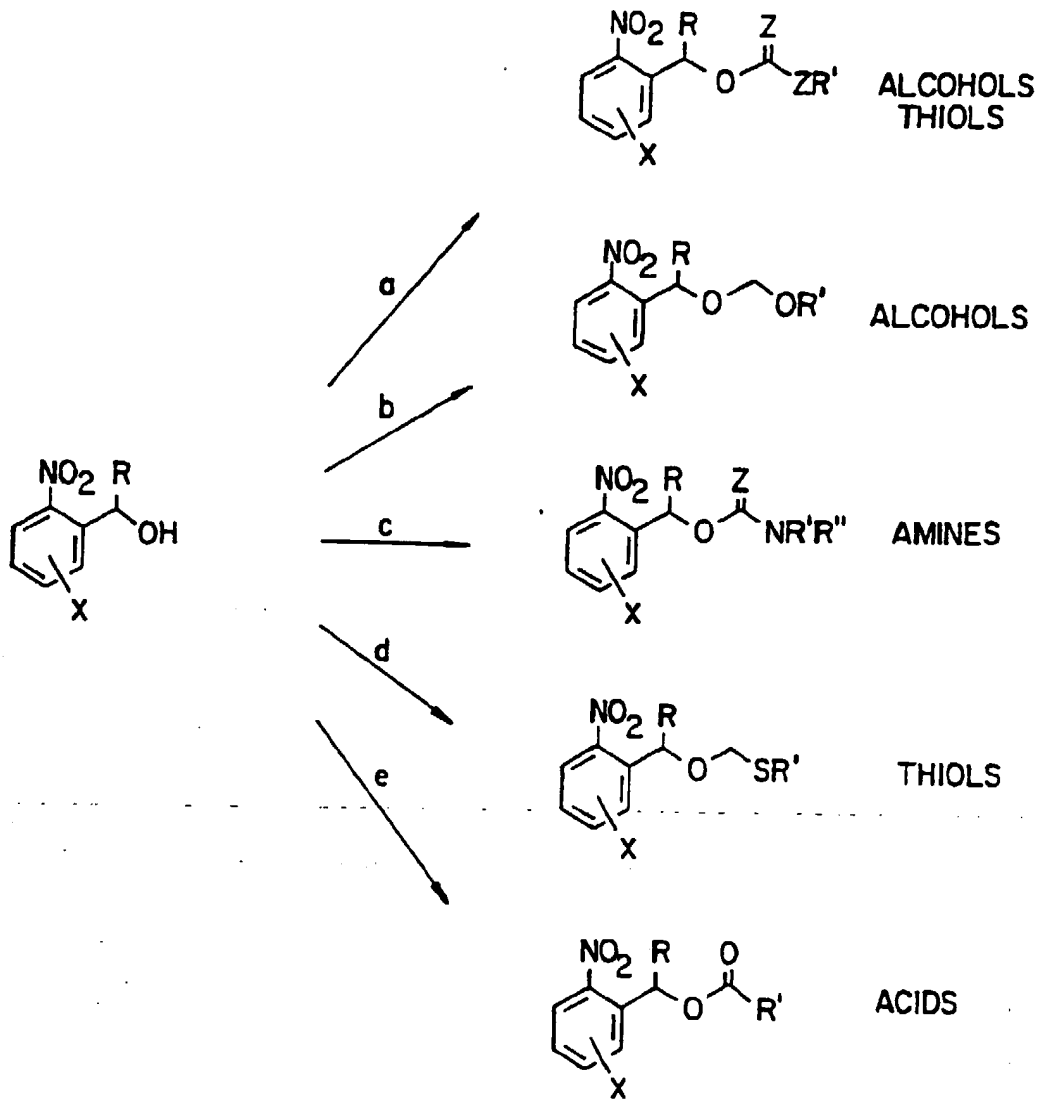

NO2 R Z
O ZR'
X
ALCOHOLS
THIOLS
a
NO2 R
O OR'
X
ALCOHOLS
b
NO2 R
OH
X
c
NO2 R Z
O NR'R"
X
AMINES
d
NO2 R
O SR'
X
THIOLS
e
NO2 R O
O R'
X
ACIDS

FIG. 2.

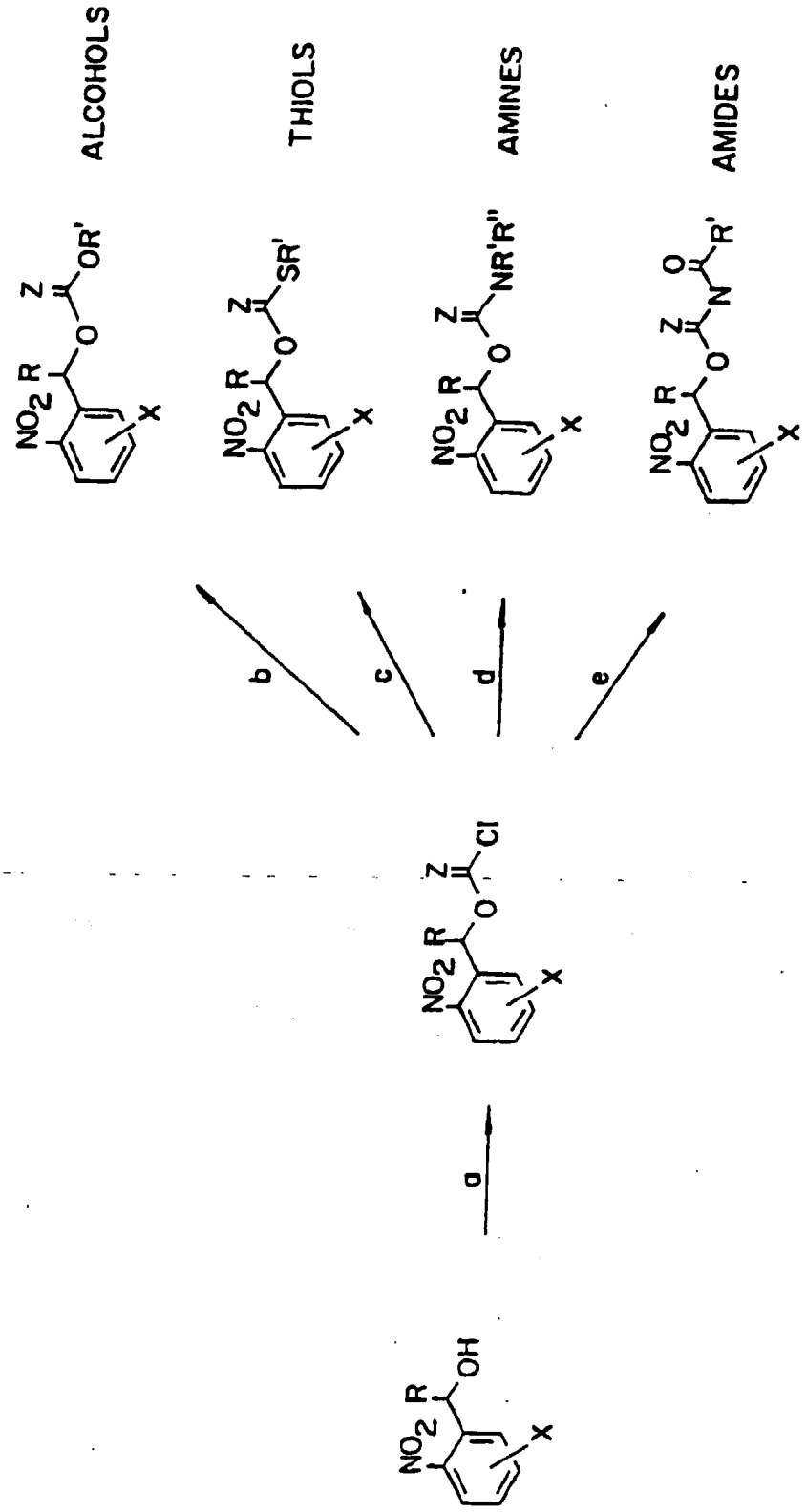


FIG. 3.

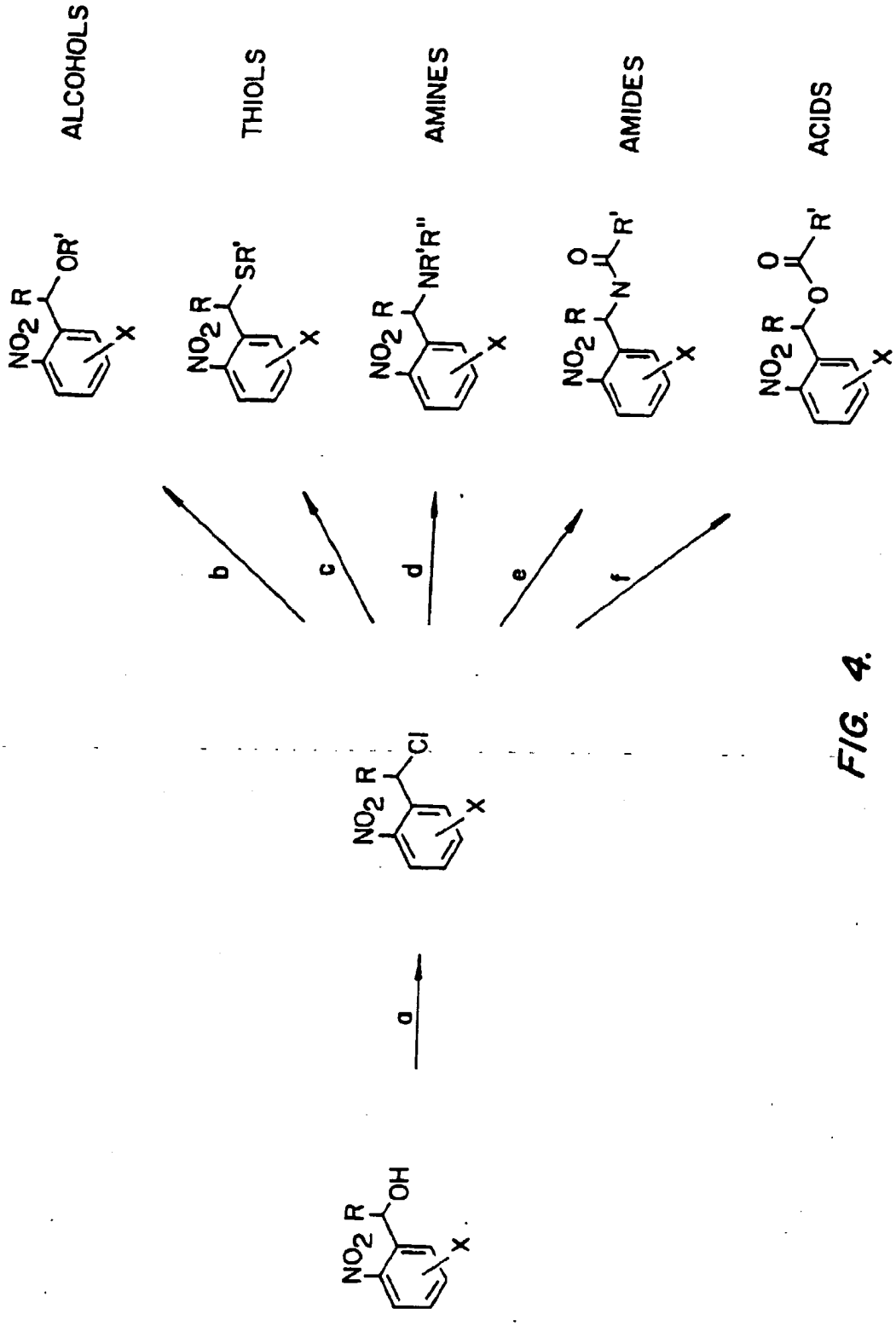

ALCOHOLS
THIOLS
AMINES
AMIDES
ACIDS
OR'
SR'
NR'R''
R'
R'
NO2
R
X
a
b
c
d
e
f
Cl
OH

FIG. 4.

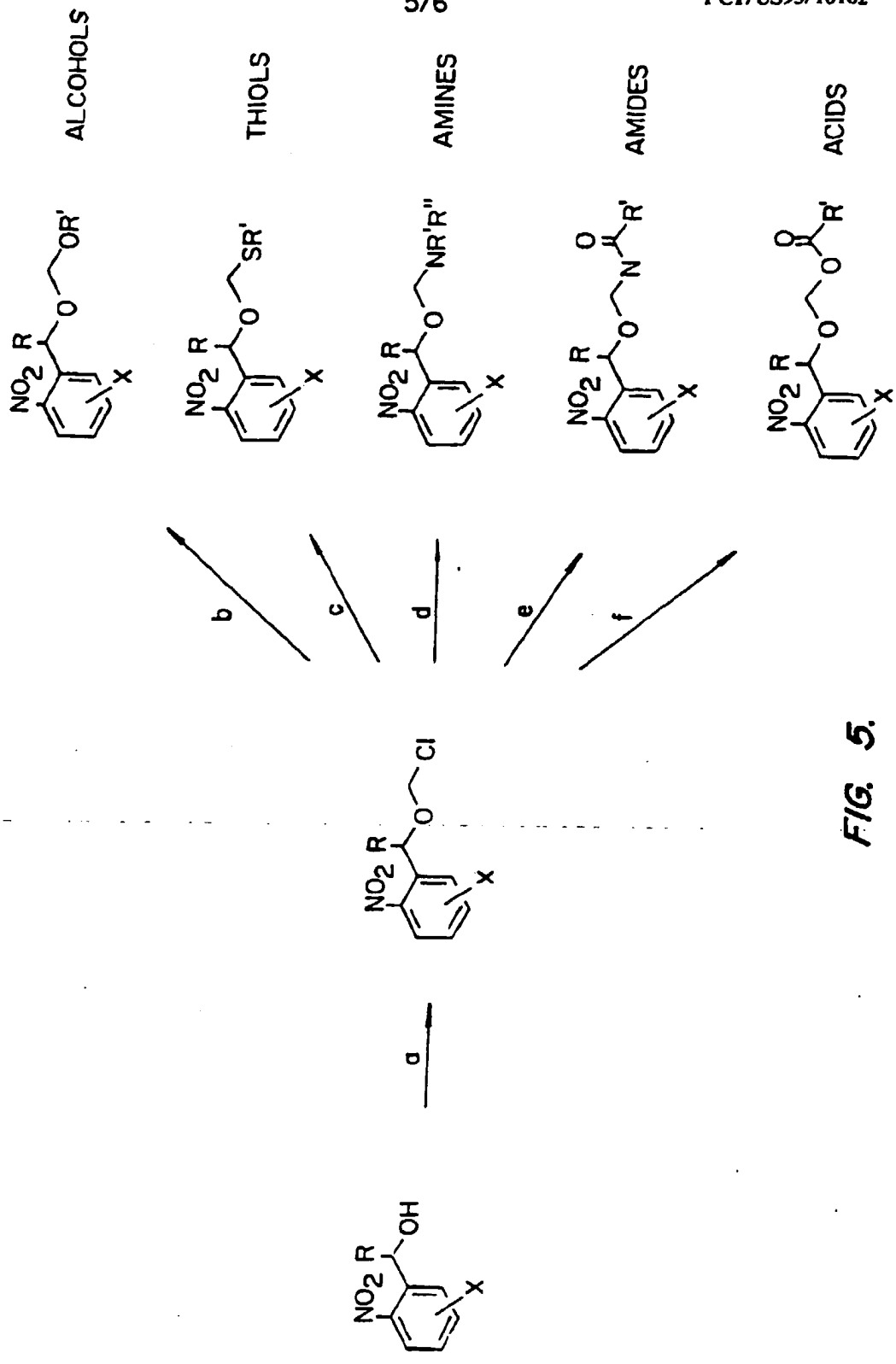


FIG. 5.

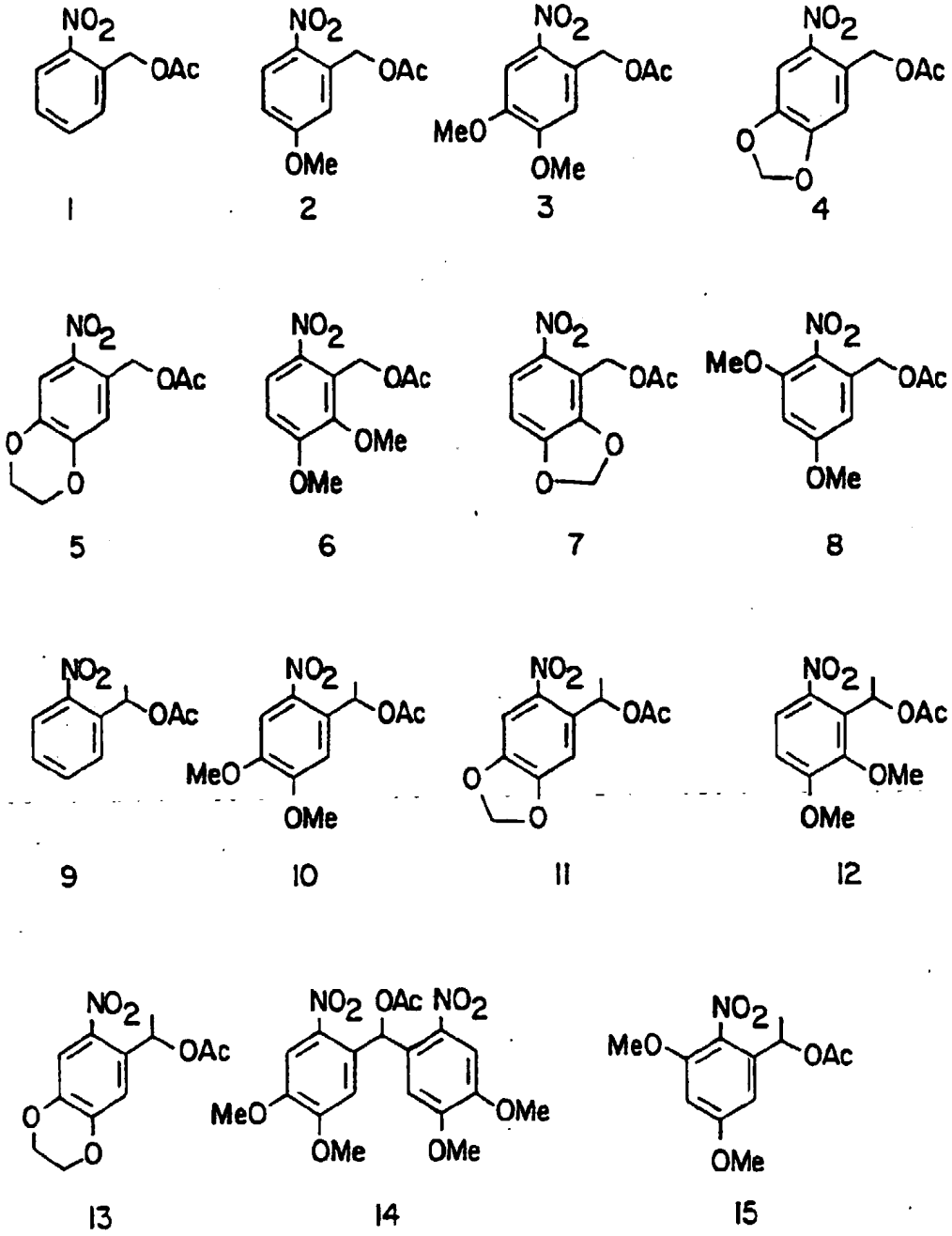


FIG. 6.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10162

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :544/243,268,316; 549/336,439,473; 568/583,584,587,588
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/243,268,316; 549/336,439,473; 568/583,584,587,588

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A, 5,143,854 (PIRRUNG ET AL) 01 SEPTEMBER 1992, see columns 12-13.	1-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 NOVEMBER 1993	Date of mailing of the international search report 07 FEB 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JOHN PEABODY Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10162

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07C 205/06,205/10,205/19,205/34; C07D 239/22,307/18,317/48,319/18; C07F 9/02